UTILIZING PARNASSIUS CLODIUS BUTTERFLIES AND NECTAR PLANT SPECIES TO EVALUATE ECOLOGICAL RESPONSES TO CLIMATE CHANGE

by

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A dissertation submitted in partial fulfillment
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DEDICATION

I dedicate my dissertation to all the young scientists. Keep being curious.

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ABSTRACT

Climate change is a pressing global issue leading to species declines, altering species interactions. Changing environmental conditions result in species adaptation or movement to suitable areas. High elevations and latitudes are susceptible to rapid change, potentially leaving no refuge for species. Plants and pollinators are particularly vulnerable to changing environmental conditions with their life cycles dependent on the timing of environmental cues that initiate species morphology, physiology, and behavior patterns. A disruption in a species' typical life-cycle pattern may lead to phenological shifts affecting biological events potentially inducing phenological mismatches between interacting species. This research addresses (1) how current and predicted environmental conditions affect the population growth rate of *Parnassius clodius* butterflies, (2) investigates the effect of warming and advanced spring snowmelt on arrowleaf balsamroot phenology and morphology, and (3) understands the effect of warming on the phenology, morphology, and photosynthetic performance of three prominent native flowering species in the Rocky Mountain Region. A mark-recapture-release program paired with life-stage hypothesis modeling was used to evaluate population growth rates of *Parnassius clodius*. Plots with plant species of interest were experimentally manipulated (warming, snow removal) and phenology, morphology, and photosynthetic performance of individuals monitored and evaluated using mixed-effects models. Findings from this dissertation determined that (1) extreme maximum temperatures during the spring led to decreased population growth rates of *Parnassius clodius* butterflies, (2) heating via passive warming and advanced spring snowmelt via snow removal led to earlier flower onset, extended flowering durations, and more flowers produced by arrowleaf balsamroot, and (3) there was no real effect of our heating treatment, but rather species-specific phenological, morphological, and photosynthetic performance differences with extended flowering durations among yarrow and geranium, more flowers produced by geranium, the greatest photosynthetic performance by yarrow followed by geranium, and maximum soil moisture, soil temperature, air temperature, and surface temperature were the best predictors of photosynthetic performance. Additionally, an iBook science communication tool was produced. These findings suggest that floral communities are faring better under a changing climate with more sensitive butterfly counterparts facing declines. Overall, this could lead to cascading effects on floral populations due to declining butterfly populations.

INTRODUCTION

Climate change is a growing issue leading to species declines, altering species interactions globally (Valiente-Banuet et al. 2015). In montane ecosystems, these changes often pressure plants and animals to adapt or move up in elevation and/or latitude (Crozier 2004, Aguirre-Gutiérrez 2016). For example, Parmesan & Yohe (2003) found that for 99 species examined globally, ranges had shifted poleward an average of 6.1 km per decade. These shifts are further exacerbated in subalpine and alpine environments, potentially leaving species no refuge from a changing climate. Species distribution shifts like this can have dramatic effects on community and ecosystem dynamics and functions (Fontaine et al. 2006).

Changes in the timing of environmental cues that initiate species morphology, physiology, and behavior patterns may lead to phenological shifts in a species' typical life cycle pattern affecting biological events and potentially inducing phenological mismatch between interacting species (Walther et al. 2002, Parmesan and Yohe 2003, Cleland et al. 2007, Scaven and Rafferty 2013). A meta-analysis found that for 172 species of plants, birds, butterflies, and amphibians, there was a mean shift of 2.3 days per decade of earlier spring timing (Parmesan and Yohe 2003). Even seemingly minute shifts like this can have cascading effects on community dynamics by shifting species abundance and distribution (Parmesan and Yohe 2003) and have the potential to lead to phenological mismatches between species (Visser and Both 2005). Phenological mismatches between plants and pollinators are occurring rapidly because both taxonomic groups are especially vulnerable to environmental shifts (Cleland et al. 2007, Memmott et al. 2007), and such changes can have lasting effects on the overall ecosystem (Burkle and Alarcon 2011).

The Greater Yellowstone Ecosystem (GYE) is a relatively intact temperate ecosystem, home to many species of mammals, amphibians, reptiles, fishes, plants, and insects that are facing the effects of a changing climate. In particular, the GYE has over 120 species of butterflies. Butterfly lifecycles are directly linked to seasonality changes, and they show immediate responses to their environment with many species completing their lifecycle within one year. These butterfly species are important bioindicators that can be used to observe and monitor the effects of climate change (Kevan 1999). One species of particular interest in the GYE is *Parnassius clodius*, a non-migratory butterfly specialized to living in subalpine and alpine meadows and dependent on only a few flowering plant species found in high elevation and high latitude environments. *Parnassius clodius* populations in the GYE have been studied since 1992 and involve ongoing collaborations with Canadian and European researchers examining other *Parnassius* species (Auckland et al. 2004, Nakonieczny et al. 2007, Roland and Matter 2013, Filazzola et al. 2020). With a changing climate, these butterflies may be forced to seek refuge at higher elevations, potentially risking displacement to areas where the resources they depend on are not available. *Parnassius clodius* are an important climatic indicator because a) their lifecycles are dependent on environmental cues and changes in seasonality, b) they are well studied, c) they are widespread across the GYE, and d) they live in small populations with low fecundity, making their populations susceptible to change. For these reasons, *Parnassius* butterflies may serve as a "canary in a coalmine" of how climate change may be affecting ecosystems where they reside. They also expand our understanding of plant-pollinator interactions that provide ecosystem services with economic and social implications to ecosystem health in the GYE.

Another species of interest is arrowleaf balsamroot (*Balsamorhiza sagittata),* a vital early-spring floral resource for pollinators in montane regions just after snowmelt. In addition to its role as a nectar source for many generalist pollinators, *B. sagittata* is a valuable source of nectar for the butterfly *Parnassius clodius* (Sherwood et al. 2017). Additionally, it serves as a critical nectar source for some specialized bee species in the genus *Osmia*, which forage exclusively on *Balsamorhiza* and *Wyethia* species (Cane 2011). Therefore, *B. sagittata*'s demography, phenology, and flower production are important for the stability of pollinator community dynamics in montane regions in parts of the western United States in early spring.

Additional species of interest in the GYE include yarrow (*Achillea millefolium*), sticky geranium (*Geranium viscosissimum*), and littleflower penstemon (*Penstemon procerus*). These species are native and abundant flowering plants in the western United States, that vary in morphology from one another, and are important for pollinator communities in the western United States (Bauer 1983, Scheinost and Stannard 2010, Krampien and Low 2020, Glenny et al. 2022, Mann et al. 2022). Therefore, evaluating the floral phenology, morphology, and photosynthetic performance responses to a warming climate will aid in understanding how flowering plants in the western United States will cope and respond to climate change. This has cascading implications on species that interact with and rely on these prominent floral resources providing valuable information on community dynamics of project climatic scenarios.

The research presented in this dissertation focuses on investigating the following questions:

(1) How does the interannual variation in population size of *P. clodius* butterflies in Grand Teton National Park, Wyoming, USA correlate with weather variables assumed to affect *Parnassius* butterflies within specific life stages (Chapter 2)?

(2) How do treatment effects of heating, advanced spring snowmelt, and heating with advanced spring snowmelt affect arrowleaf balsamroot flower onset, flowering duration, maximum floral display, the cumulative treatment effects over time, and the correlations among plant responses to treatments to understand how flower onset, flowering duration, and maximum floral display are associated with one another in Grand Teton National Park, Wyoming, USA (Chapter 3)?

(3) How does heating affect the photosynthetic performance, phenology, morphology, the relationships between them, and the effect of local abiotic environmental conditions on photosynthetic performance of yarrow, sticky geranium, and littleflower penstemon in the Rocky Mountain Region of the USA (Chapter 4)?

The final chapter (i.e., Chapter 5) of this dissertation is not an investigative research report, but rather a science communication tool highlighting *Parnassius clodius* butterflies and their connection to their environment and other organisms in Grand Teton National Park, Wyoming, USA. The tool encompasses a free downloadable interactive iBook promoting observation and public scientist efforts (i.e., anyone participating in the scientific process) along with teaching lessons covering phenology and morphological adaptations written for the general public, ages nine and up. Activities are associated with each lesson and include how to build your own field guide, building a butterfly character card, and phenological tracking over time.

The research included in this dissertation helps address how environmental conditions over time affect (1) the life stages of the montane butterfly, *Parnassius clodius*, reflected in population growth rates, (2) the phenological and morphological responses of an early-season native flowering plant, arrowleaf balsamroot, to predicted warming and advanced spring snowmelt, (3) the phenological, morphological, and photosynthetic performance responses of three prominent native flowering plants (i.e., yarrow, sticky geranium, and littleflower penstemon) to heating, and (4) science communication of environment-plant-pollinator interactions and public engagement in the scientific process. Answers to these knowledge gaps will improve our understanding of how native flowering plants and a butterfly species will respond to predicted climate change scenarios with implications to community and ecosystem dynamics.

CHAPTER TWO

MONTANE BUTTERFLY DEVELOPMENTAL LIFE STAGES AS INDICATORS OF INSECT VULNERABILITY TO EXTREME CLIMATIC CONDITIONS

Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 1

Author: J. Simone Durney

Contributions: contributed to data collection, conducted analyses, and was the primary author drafting the manuscript for publication

Co-Author: Diane M. Debinski

Contributions: received funds for research, conducted data collection and contributed to drafting manuscript for publication

Co-Author: Stephen F. Matter

Contributions: consulted on data analysis methodology and contributed to drafting manuscript for publication

Manuscript Information

J. Simone Durney, Diane M. Debinski, and Stephen F. Matter

Oikos

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☒ Officially submitted to a peer-reviewed journal
☐ Accepted by a peer-reviewed journal
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Abstract

Butterflies are important bioindicators that can be used to monitor the effects of climate change, particularly in montane environments. Changes in butterfly population size over time can signal changes that have occurred or are occurring in their environment indicating ecosystem health. From the perspective of understanding butterflies as bioindicators in these systems, it is essential to identify influential environmental variables at each life stage that have the greatest effect on population dynamics. Life stage hypothesis modelling was used to assess the effects of multiple temperature and precipitation metrics on the population growth rate of a *Parnassius clodius* butterfly population from 2009 to 2018. Extreme maximum temperatures during the larval-pupal life stages were identified to have a significant effect on population growth rate, leading to a decline in the size of the population's next generation. We hypothesize that higher temperatures during the spring ephemeral host plant's flowering, and *P. clodius*'s larval stage together, may lead to earlier plant senescence and lower *P. clodius* growth. Because *Parnassius* butterflies are well studied from a global perspective, our results may aid in understanding the potential vulnerabilities of other insect species in montane environments to climatic changes. Findings from this study demonstrate the value in assessing a butterfly species' response to short-term weather variation or long-term climatic changes *at each life stage* in order to protect and conserve insects and their interactions with other organisms.

Introduction

Insects are responding to climate change globally (Parmesan et al. 1999, Parmesan and Yohe 2003, Diamond et al. 2011, Forister et al. 2021) – sparking a need to investigate how

climate affects insect population dynamics. It is becoming increasingly crucial to identify influential weather variables and climatic patterns that play a role in insect population change and distributional shifts (Roland and Matter 2016). The term weather refers to atmospheric conditions for a specific time and place changing hourly, daily, and seasonally, while climate is a statistical characterization of weather represented by means, variability, and extremes over many years (Moore et al. 2010). Once identified, these influential weather variables and climatic patterns may help in predicting population changes that could result from either short-term weather variation or long-term climatic changes (Roland and Matter 2016) and reflect greater changes occurring in the environment (Samways et al., 2010).

Insects serve as bioindicators because (1) they are highly sensitive to local environmental conditions due to their small size, (2) they move towards optimal conditions due to mobility and reactivity to changing conditions, (3) their abundance is highly responsive to short- and long-term change due to short breeding rates and generation times, (4) they can be linked to specific environmental variables due to different growth rates, life history traits, body sizes, food preferences, and ecological preferences, and (6) they are relatively easy to survey (Samways et al., 2010). Montane insect species, i.e., those associated with mountain environments, can serve as bioindicators for the effects of climate change in higher elevations and latitudes, because their species distribution and abundance patterns reflect the subtle changing conditions of these high-elevation environments (Samways et al., 2010).

A broad set of insect taxa have been documented to respond to climatic changes in recent decades. For instance, in response to changing environmental conditions (i.e., climate change & habitat modification), northern, cold-adapted moth species declined, while southern, warm-

adapted moth species grew in abundance in Great Britain over a 40-year period (Fox et al. 2014, Halsch et al. 2021). Warmer winters in the northeastern U.S. over 45 years drove an 83% decline in beetle abundance and a 39% decrease in number of beetle species captured, with reduced snow cover depths and duration associated with fewer beetles captured the following growing season (Harris et al. 2019, Halsch et al. 2021). Alpine grasshopper communities over 30 years in the northern Alps displayed expansion and increased densities of generalists, while specialists declined in response to warmer and dryer conditions (Illich and Zuna-Kratky 2022). A German headwater stream within a protected area experienced an 82% decline in aquatic insect abundance over 45 years associated with warmer water temperatures and altered discharge patterns (Baranov et al. 2020, Halsch et al. 2021). When occupancy and richness of 66 bumble bee species across North America and Europe were compared between 1901-1974 and 2000-2014, occupancy declined on average by 46% in North America and 17% in Europe, while species richness declined on average by 20% in North America and 35% in Europe. Both bumble bee metrics declined relative to increasing frequency of high temperatures (Soroye et al. 2020). These long-term insect datasets demonstrate that insect distribution and abundance patterns, across a broad range of orders and families, can serve as bioindicators for climate change in montane ecosystems across various environmental stressors.

Butterflies in particular are important bioindicators that can be used to monitor the effects of climate change over time (Kevan 1999, Diamond et al. 2011). As an easily surveyed, small ectothermic organism with a rapid generation time, butterflies are an ideal model organism to evaluate biodiversity and shifts in species distributions geographically in response to weather and climate variation (Kevan, 1999; Samways et al., 2010). Changes in butterfly population size over

time can be a strong signal of changes that have occurred or are occurring in their environment (Kevan 1999, Horn 2003). Suitable butterfly habitat is decreasing globally due to human land use intensification, landscape-scale degradation due to pollution, and climate change, leading to declining butterfly population sizes and shifting range distributions in North America, the U.K. and other locations (White and Kerr 2006, 2007, Thomas 2016, Forister et al. 2021). The abundances of butterfly species in North America and Europe have been declining during the last half century in response to habitat changes due to warming and drying (Forister et al. 2021), habitat changes from direct anthropogenic influences (Nakonieczny et al. 2007, Boggs and Inouye 2012), and distributions have shifted poleward and to higher elevations in response to declining suitable habitat (Parmesan et al. 1999, Hill et al. 2002, Parmesan and Yohe 2003, Condamine and Sperling 2018). Butterflies that persist in small, isolated populations with specific habitat requirements are at greater risk of decline or extinction compared to other butterfly species (Szcodronski et al. 2018). For example, the critically imperiled Fender's blue butterfly (*Icaricia icarioides fenderi*) exists in small, isolated patches of Oregon prairie where <0.5% of its original habitat contains its larval host plant, Kincaid's lupine (*Lupinus sulphureus kincaidii*) (Schultz 2001, Schultz and Crone 2005). Further, butterfly phenological responses are advancing three times stronger than their plant counterparts (Parmesan 2007) in response to climate change.

Identification of influential weather variables and climatic patterns at each butterfly life stage is needed so that scientists and managers can prioritize butterfly conservation efforts during the most vulnerable life stage in the most threatened geographic locations. A study conducted over 17 years in two mountainous regions of the Czech Republic tested the effect of climatic

conditions during two larval phases (i.e., fall/spring activity and overwintering) on subsequent adult abundances of two *Erebia* butterflies (*E. epiphron* and *E. sudetica*) to evaluate which weather and climate variables drive population dynamics of these montane insects (Konvicka et al. 2021). Decreased precipitation and warmer temperatures during overwintering were associated with decreased adult abundances, while increased adult abundances were observed with warmer temperatures in the fall and warmer and drier springs during active larval phases (Konvicka et al. 2021). Additionally, higher minimum temperatures during the adult flight season were associated with decreased butterfly abundances (Konvicka et al. 2021). This research demonstrates that evaluating life stages may identify vulnerabilities of bioindicator insect species life cycles to weather patterns. A meta-analysis found a 1.6% annual reduction in the number of individual butterflies in the western US over the past 40 years in response to warmer fall temperatures, with single brood (univoltine) species declining more severely (Forister et al. 2021). Conversely, more butterflies were observed in response to warmer temperatures during summer months in some western US areas and in response to increased precipitation associated with positive effects on interacting plant species (Forister et al. 2021). These research projects detail the positive and negative effects that short-term weather variation or long-term climatic changes can have on butterfly species. Observational research conducted over 15 years in Alberta, Canada investigated the direct and indirect effects of various weather variables on sub-populations of *Parnassius smintheus* to predict their response to a changing climate (Matter et al. 2011, Filazzola et al. 2020). Direct effects included increased overwintering mortality in response to extreme minimum temperatures reaching -27.9°C and frequent loss of snow cover, and premature larval development under elevated temperatures, associated with earlier

emergence which negatively affected survival and population growth (Matter et al. 2011, Filazzola et al. 2020). Indirect effects included temperature and precipitation changes affecting growth and survival of host and nectar plant species limiting larvae and adult resources, thus affecting butterfly distribution (Matter et al. 2011, Filazzola et al. 2020). Due to the increasing frequency of short-term extreme weather events, such as extreme temperature, precipitation, and natural disasters (van Aalst 2006), and longer-term changes in variables such as minimum and maximum daytime and nighttime temperatures, annual snowpack, etc. (Whitlock et al. 2017, IPCC 2021), understanding these relationships will become even more important in the future.

Parnassius butterflies serve as model species for insect conservation in montane ecosystems across the globe (Nakonieczny et al. 2007, Todisco et al. 2010). *Parnassius* are non-migratory butterflies, making them susceptible to short-term weather variation and the long-term effects of climate change. These butterflies live in local populations with limited fecundity, making their populations susceptible to change. *Parnassius* are restricted to high elevation montane meadows, environments predicted to be at greater risk and expected to undergo rapid change under a changing climate (Kim et al. 2002, Thuiller et al. 2005, Nakonieczny et al. 2007, Roland and Matter 2013). Further, these butterflies' lifecycles are intricately dependent on the climatic patterns associated with seasonality in montane meadows. Changing climate patterns will likely lead to earlier larval emergence due to prolonged warm weather in the fall and/or spring (Crees and Debinski 2021), altering butterfly life stage timing inducing mismatch between larval emergence and host plant emergence and availability (Calabrese et al. 2008, Crees and Debinski 2021).

Here, we analyzed the population dynamics of a *Parnassius clodius* population in Grand Teton National Park, WY, USA from 2009 to 2021 in relation to weather and climate data to assess how this species is responding to changing climate in a montane region. Populations of *P. clodius* are well distributed in the Teton region, with 78% of suitable surveyed meadows occupied in 2013 (Szcodronski et al. 2018). However, many of the monitored populations had low numbers of individuals. Like many insect species, *P. clodius* population dynamics have yet to be evaluated in determining their conservation status. *Parnassius clodius* is currently not threatened or endangered in its native range of North America. However, it faces similar threats as its European sister species, *Parnassius apollo*, which is listed on the IUCN red list of threatened species due to habitat loss from forest encroachment, climatic changes, heavy metal contamination, over collection, and genetic erosion (Nakonieczny et al. 2007). By studying how *P. clodius* population dynamics are correlated with interannual variation in weather variables, we may be able to identify similar factors affecting other *Parnassius* species and predict how these factors may impact *P. clodius* in the future. The goal of this research was to evaluate how the interannual variation in population size of *Parnassius clodius* was correlated with weather and climate variables assumed to affect *Parnassius* butterflies within specific life stages. Weather conditions during the overwintering period (i.e., egg life stage) were hypothesized to have the greatest effect on population size compared to the larval/pupal and adult life stages based on previous findings evaluating population growth of *Parnassius smintheus* (Matter et al. 2011).

Materials and Methods

Species Description

Parnassius clodius (Lepidoptera: Papilionidae) is a medium-sized butterfly that is mostly white with a hairy yellow body and inhabits western Canada and U.S.A. (Figure 1). They are often found in open montane woodlands and meadows but can extend to higher elevations and reside on mountaintops. Their host-plant is steer's head (*Dicentra uniflora*) (Fumariaceae) (Scott, 1986), a small herbaceous perennial and a spring ephemeral (Crees & Debinski, 2021; Scott, 1986). The primary nectar source of *P. clodius* in this region is sulphur flower buckwheat (*Eriogonum umbellatum*) (Auckland et al. 2004) along with several other flowering plant species (Szcodronski et al. 2018, Crees and Debinski 2021). Previous research revealed suspected positive associations between *P. clodius* occupancy and low sagebrush (*Artemisia arbuscula*) and *Dicentra uniflora* (i.e., *P. clodius*'s host plant), with the presence of big sagebrush (*Artemisia tridentata*) and lupine (*Lupinus* spp.) having a clear negative association with *P. clodius* occupancy (Szcodronski et al. 2018).

Parnassius clodius is a univoltine species that overwinters as a pharate first instar larvae (i.e., egg) (Scott, 1986). Following spring snowmelt, the larvae emerge to feed and take two to three weeks to mature (Crees and Debinski 2021). Larvae form a chrysalis on the ground typically among rocks and debris to pupate over a one-to-two-week period. Typically, adults emerge from pupae in mid to late June with males emerging prior to females. Peak flight is usually late June to early July in Grand Teton National Park, although this can vary by several weeks, depending upon spring conditions (pers. obs.). *Parnassius clodius* females oviposit on vegetation, such as sagebrush and the undersides of balsamroot leaves, near host plants for the

remainder of the flight season, which often ends in mid-July (Crees and Debinski 2021). Microclimatic conditions influence the timing of when each life stage takes place (i.e., the phenological progression) (Crees and Debinski 2021).

Study Area

The study area (Pilgrim Creek) was a relatively homogenously vegetated sagebrush meadow with dry, gravelly soils surrounded by coniferous woodlands along the meadow edge in Grand Teton National Park, Wyoming, USA at 43.915915°N, -110.578850°W and 1500m X 300m in size. The meadow is relatively flat, and at an elevation of 2120 m. The vegetation community of the meadow included mostly low sagebrush (*Artemisia arbuscula*), some big sagebrush (*Artemisia tridentata*), several known *P. clodius* nectar plant species listed in the *species description* section, and *P. clodius'* host plant – *Dicentra uniflora*.

Butterfly Mark-Recapture

Mark-recapture methods were used to study a population of *Parnassius clodius* at Pilgrim Creek across annual flight seasons between 2009 and 2018 during June and July. Surveys were not carried out in 2012 and 2013. Six 50m x 50m plots a minimum of 100m apart were located using GPS units, flagged prior to the flight season of *P. clodius*, and surveyed each year. Mark-recapture surveys began a few days after the beginning of the flight season and continued until only one or two butterflies per plot were caught during a survey period. Plots were monitored daily if weather permitted throughout each flight season. Surveys were conducted when temperatures were above 21°C, wind was $<16 km h^{-1}$, and clouds were not obscuring the sun. If weather prevented all six plots from being sampled in one day, the rotation was completed the

following day. The order in which plots were surveyed rotated throughout the flight season to vary the time of day surveys were conducted for each plot.

Two people surveyed each plot for 20 minutes between 10:00 and 17:00 hours. All *Parnassius* butterflies in a plot were netted by hand and held in glassine envelopes until the survey ended. The time of capture, sex, and activity of the butterfly when captured (i.e., nectaring, chasing, stationary) were noted. Captured butterflies were marked with a permanent marker on each hindwing with an identification number indicating the plot it was caught in and the butterfly's individual number (Figure 1). The number corresponded to the consecutive butterfly count throughout a season. All butterflies were released in the center of each plot by placing butterflies on surrounding plants, rather than being placed on the ground where they could be stung by ants.

Weather and Climate Variable Selection

A list of meaningful weather variables and climatic patterns expected to affect populations of *Parnassius clodius* butterflies was formulated based upon previous research that identified influential weather and climate variables affecting the population dynamics of other *Parnassius* species (Matter et al. 2011, Roland and Matter 2013, 2016, Matter and Roland 2017) as well as our observations at Pilgrim Creek over time. The list of explanatory variables included: date of snowmelt, maximum snow water equivalent (cm), mean snow water equivalent (cm), mean temperature (C), mean maximum temperature (C), extreme maximum temperature (C), mean minimum temperature (C), extreme minimum temperature (C), accumulated rainfall (cm) from 2008 to 2018 (Table 1). All temperature data were sourced from the USDA Base Camp SNOTEL site located at 43.93333°N, -110.45000°W at 2151 m in elevation and 10.7 km

from Pilgrim Creek meadow. All snow water equivalent and precipitation data were sourced from the USDA Snake River Station SNOTEL site located at 44.13333°N, -110.66667°W at 2109 m in elevation and 25.1 km from Pilgrim Creek meadow.

Analysis

The goal of these analyses was to identify which weather variables, known to affect other butterfly species, explain variation in *Parnassius clodius* population growth through each life stage. Estimates of population change were calculated for the Pilgrim Creek population of *P. clodius* for the duration of the study (i.e., 2009-2011, 2014-2018). Calculations of population change (R_t) were estimated as the ratio of the number of caught butterflies (N_t) for one flight season after accounting for effort, relative to the number of caught butterflies of the previous flight season after accounting for effort. Effort was measured as the number of two-person 20-minute plot surveys conducted during each flight season. R_t was calculated as $\log_{10}((N_{t+1}/effort_{t+1})/(N_t/effort_t))$ (Roland and Matter 2016). Our estimate of population size was included in each model to evaluate density dependence as an explanatory variable on population growth. Population size was estimated by taking the log of the number of caught butterflies divided by the number of surveys conducted for each flight season (i.e., $\log_{10}(N_t/effort_t)$).

A life stage hypothesis modeling approach was used to evaluate the effect of each chosen weather variable during each life stage on the population growth (i.e., R_t) of *Parnassius clodius* butterflies. The observed values for each weather variable were partitioned by life stage to evaluate each variable's effect during each stage of the *P. clodius* lifecycle. Dates of each life stage were estimated from researcher observations (L. Crees, personal communication, October 2022) of *P. clodius* over time to capture the *estimated* beginning and end dates of each life stage.

We categorized the egg stage as July 21 - April 30, the larva-pupa stage as May 1 - May 31, and the adult stage June 1 - July 20. Each weather and climate variable was tabulated for the egg stage, larva-pupa stage, and the adult butterfly stage (Table 1). The larva and pupa life stages were combined due to the uncertainty of the date of when one stage ended and the other began.

Correlation tests were conducted between all explanatory weather variables to prevent the inclusion of highly correlated variables in models, which could lead to multicollinearity and unreliable estimates. Uncorrelated precipitation and temperature explanatory variables and $\log N_t$ (i.e., density dependence) were included in each generalized linear model. Then, generalized linear models using the *glm* function in R were fit to evaluate which explanatory weather variables were the most descriptive in explaining population change (R_t). AIC comparison between models was conducted to determine the most informative explanatory variables. Generalized linear models that included interactions between the most informative temperature and precipitation explanatory variables and $\log N_t$ were used to evaluate potential interactive effects between weather and climate variables along with density dependence on population change. Final AIC comparison was conducted to determine the best model to describe population change for each *P. clodius* life stage.



Figure 1: *Parnassius clodius* butterfly. Photo Credit J. Simone Durney.

Table 1: The nine explanatory weather and climate variables tabulated by each *Parnassius clodius* life stage included in analyses.

Weather and Climate Variables Evaluated
Julian date of snowmelt
Maximum snow water equivalent (centimeters)
Mean snow water equivalent (centimeters)
Mean temperature (Celsius)
Mean maximum temperature (Celsius)
Extreme maximum temperature (Celsius)
Mean minimum temperature (Celsius)
Extreme minimum temperature (Celsius)
Accumulated rainfall (centimeters)

Results

A *Parnassius clodius* population was monitored from 2009-2018, excluding 2012 and 2013, producing eight interannual estimates of population change. During the study, 532 surveys were conducted and 3,868 *P. clodius* individual butterflies were caught. Population size and change (R_t) varied over the study with population increases occurring in 2011 and population declines occurring in 2009 (Figure 2).

Egg Life Stage

Correlation tests revealed that extreme maximum temperature was not highly correlated (i.e., $-0.5 < r < 0.5$) with the explanatory weather variables and thus was included in the full generalized linear model. Due to collinearity among the remaining weather variables, generalized linear models were constructed with population change (i.e., response variable) and each remaining explanatory weather variable (i.e., Julian date of snowmelt, maximum and mean snow water equivalent, mean temperature, mean maximum temperature, mean minimum temperature, extreme minimum temperature, accumulated rainfall) (Table 2). AIC comparison between those models was conducted to determine which weather variables were the most descriptive in explaining population change (Table 2). Thus, mean snow water equivalent, mean minimum temperature (Table 2) and extreme maximum temperature were the best descriptors of population change during the *P. clodius* egg life stage. However, including those weather variables with and without interactions along with previous populations size in generalized linear models resulted in no explanation of significant independent variation of population change during the egg life stage and did not significantly differ from the null model ($F_{(3,7)} = 4.0271$; $p = 0.1059$) (Table 3). Further, including each descriptive weather variable in its own generalized

linear model with population change as the response revealed that none of the descriptive weather variables during the egg stage significantly explained population change (Figures 3 & 4).

Larval-Pupal Life Stage

All explanatory weather variables were highly correlated during the larval-pupal life stage. Therefore, generalized linear models were constructed with population change as the response variable and each explanatory weather variable. AIC comparison determined that accumulated rainfall and extreme maximum temperature were the best descriptors (i.e., lowest AIC value) (Table 2) of population change during the *P. clodius* larva-pupa life stages. The full model including all descriptive weather variables, their interactions, and density dependence resulted in no significant explanation of independent variation on population change. The same was true for the full model excluding interactions. Each descriptive weather variable was evaluated in its own generalized linear model with population change as the response. For the larval-pupal life stage, we found that extreme maximum temperature significantly explained population change ($\beta = -0.095 \pm 0.031$(SE), $t = -3.032$, $p = 0.023$) (Figures 3 & 4; Table 3).

Adult Life Stage

Correlation tests determined that extreme minimum temperature during the adult stage was not highly correlated with the other explanatory weather variables and so was included in the full generalized linear model for the adult stage. Of the remaining variables, maximum snow water equivalent and extreme maximum temperature had the lowest AIC values during AIC comparison (Table 2). Thus, maximum snow water equivalent, extreme maximum temperature, and extreme minimum temperature were the best descriptors of population change during the *P.*

clodius adult life stage. However, including those weather variables with and without interactions along with population size in generalized linear models resulted in no explanation of significant independent variation of population change during the adult life stage and did not significantly differ from the null model ($F_{(3,7)} = 2.3194$; $p = 0.2170$) (Table 3). Including each descriptive weather variable in its own generalized linear model with population change as the response revealed that none of the descriptive weather variables during the adult life stage significantly explained population change (Figures 3 & 4).

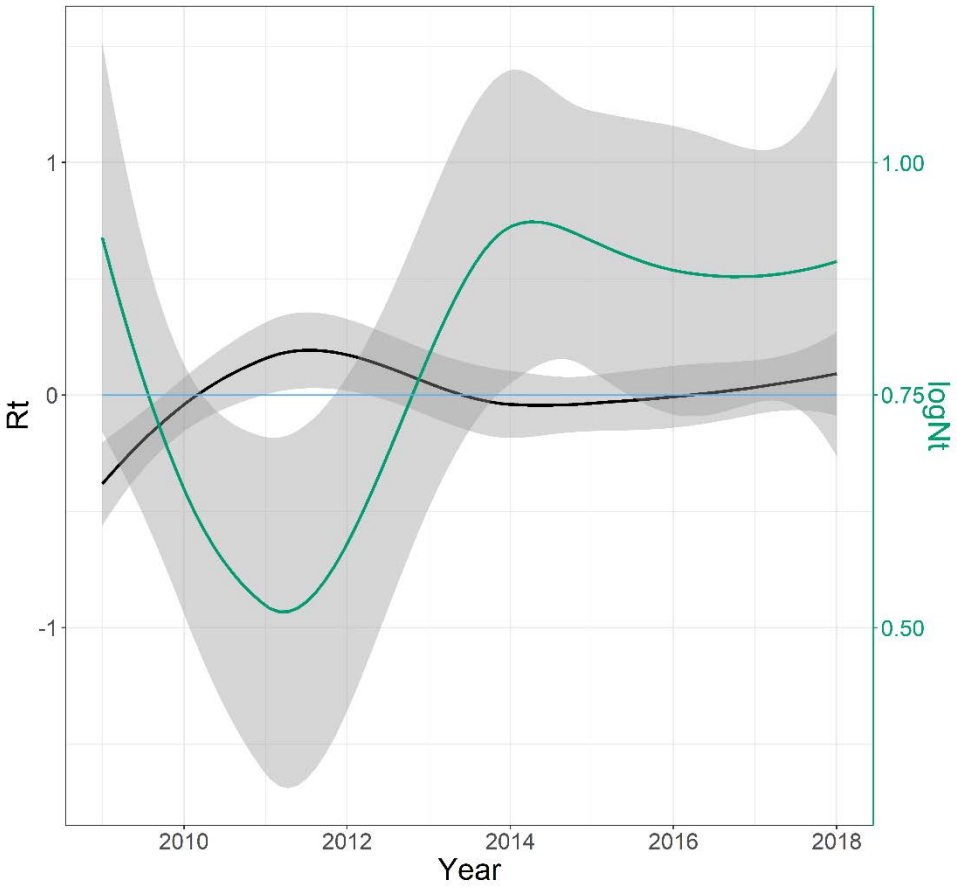


Figure 2: Years of study with associated population change (R_t) values represented in black, no population change represented by the horizontal blue line, and density dependence ($logN_t$) values represented in green. Note: surveys were not carried out in 2012 and 2013.

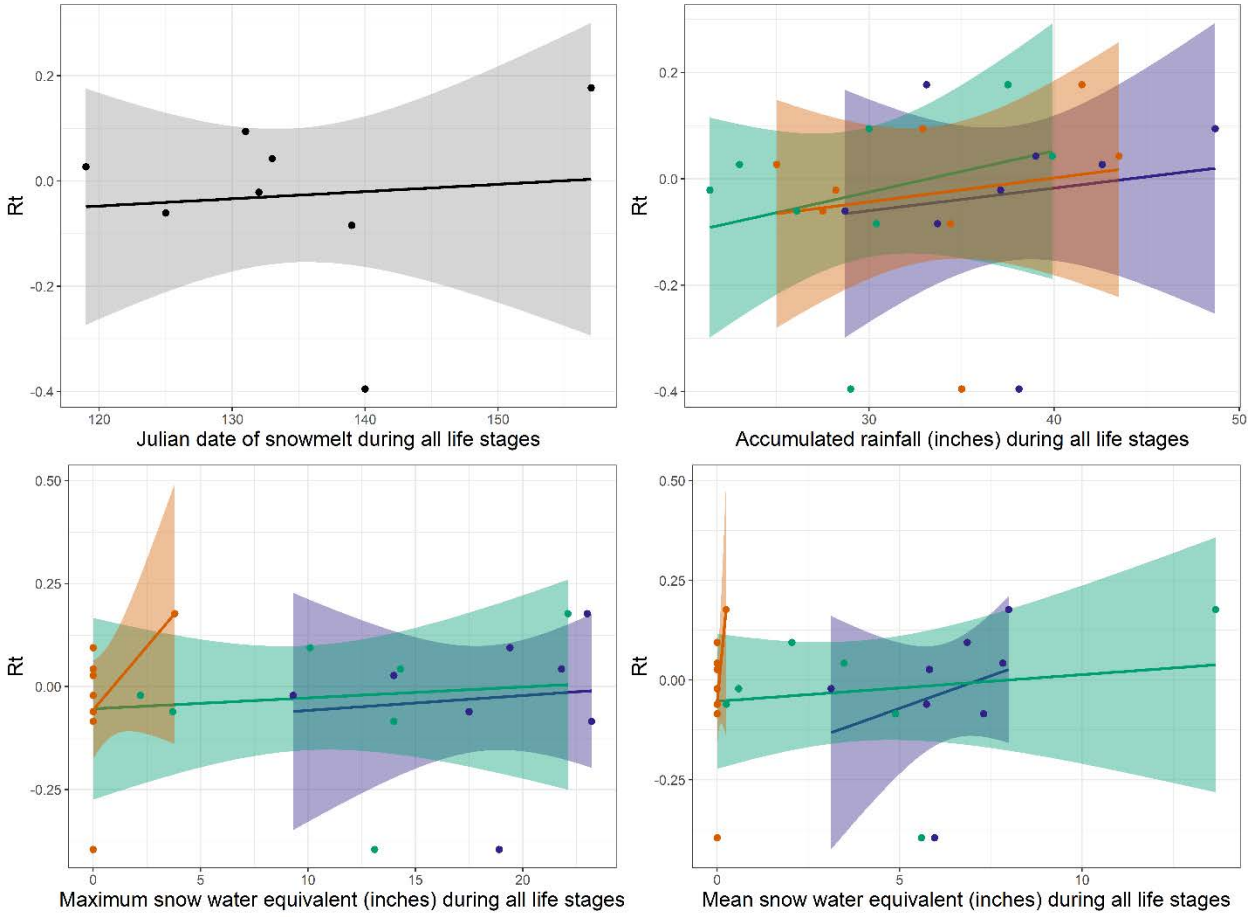


Figure 3: The relationship between *Parnassius clodius* population change (R_t) from 2009 to 2018 by life stage to the precipitation explanatory variables considered in these analyses. The egg life stage is represented by the color purple, the larval-pupal life stages are represented by the color green, and the adult life stage is represented by the color orange. Julian date of snowmelt did not vary across the life stages and is represented by the color black. Lines and points indicate a significant relationship between population change and a descriptive weather or climate variable at a particular life stage. Note: surveys were not carried out in 2012 and 2013. Overall, there were no significant relationships detected between population change (R_t) and date of snowmelt, maximum snow water equivalent (cm), mean snow water equivalent (cm), and accumulated rainfall (cm).

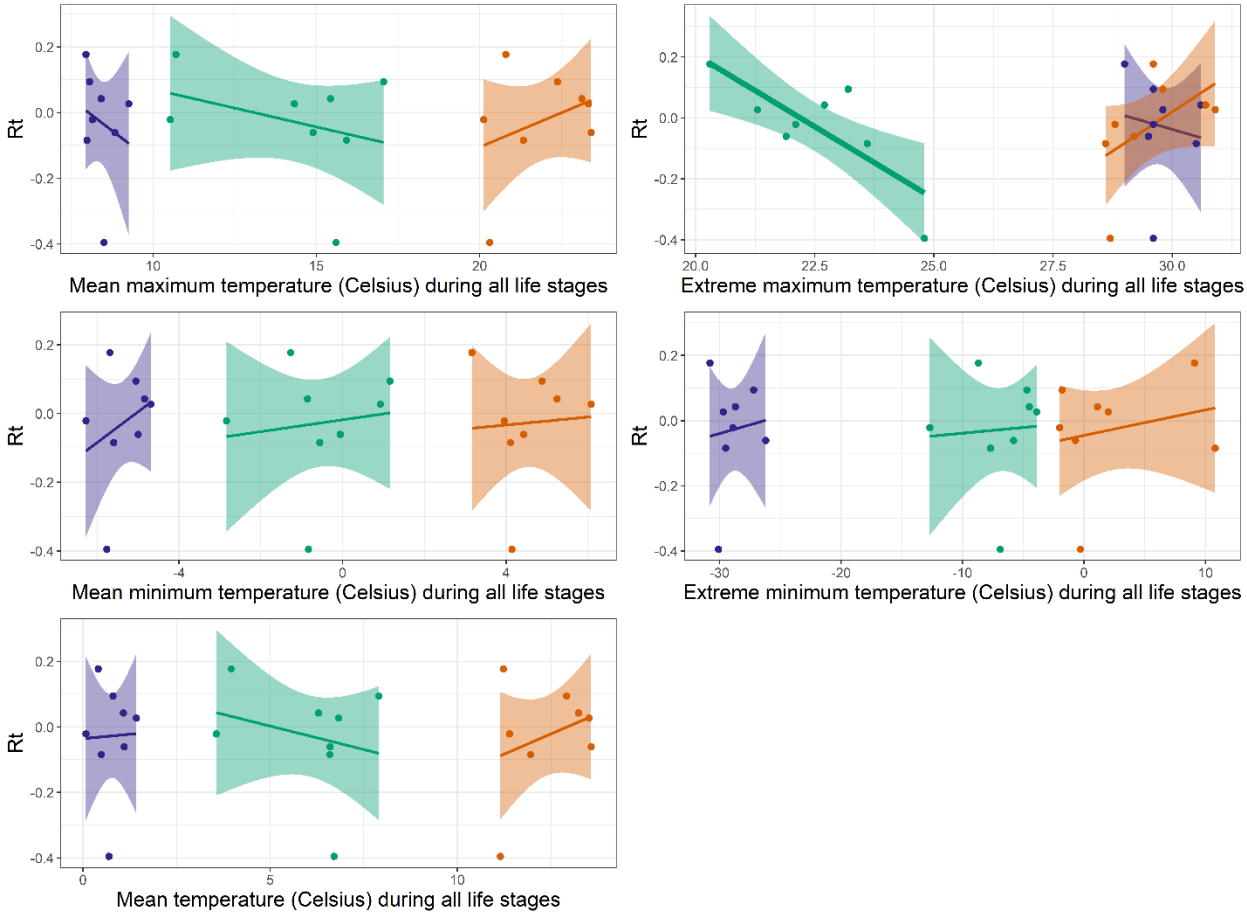


Figure 4: The relationship between *Parnassius clodius* population change (R_t) from 2009 to 2018 by life stage to the temperature explanatory variables considered in these analyses. The egg life stage is represented by the color purple, the larval-pupal life stages are represented by the color green, and the adult life stage is represented by the color orange. Darker, thicker lines and points indicate a significant relationship between population change and a descriptive weather variable at a particular life stage. Note: surveys were not carried out in 2012 and 2013. Overall, a significant negative relationship between extreme maximum temperatures during the larval-pupal life stage and population change (R_t) was detected. There were no significant relationships detected between population change (R_t) and mean temperature (C), mean maximum temperature (C), mean minimum temperature (C), and extreme minimum temperature (C).

Table 2: AIC comparison table for models testing for the best predictive weather and climate explanatory variables among highly correlated (i.e., r >0.5) variables to be included in full generalized linear models for each life stage (i.e., egg, larval-pupal, adult). The best predictive weather and climate variables for each life stage are in bold font. *Note: due to correlation between maximum and mean snow water equivalent, maximum snow water equivalent was chosen as the predictor variable during the adult life stage since the AIC values were the same between the two variable models.

Life Stage	Weather or Climate Variable Type	Model	df	AIC
Egg	Precipitation	**R_t ~ Mean Snow Water Equivalent**	3	**-1.3919**
		R_t ~ Max Snow Water Equivalent	3	-0.7237
		R_t ~ Julian Date of Snowmelt	3	-0.7088
	Temperature	**R_t ~ Mean Minimum Temperature**	3	**-1.3306**
		R_t ~ Mean Maximum Temperature	3	-0.9940
		R_t ~ Mean Temperature	3	-0.6472
Larval-Pupal	Precipitation	**R_t ~ Accumulated Rainfall**	3	**-1.3621**
		R_t ~ Mean Snow Water Equivalent	3	-0.8933
		R_t ~ Max Snow Water Equivalent	3	-0.7500
		R_t ~ Julian Date of Snowmelt	3	-0.7088
	Temperature	**R_t ~ Extreme Maximum Temperature**	3	**-8.0743**
		R_t ~ Mean Maximum Temperature	3	-1.5279
		R_t ~ Mean Temperature	3	-1.1596
		R_t ~ Mean Minimum Temperature	3	-0.7731
		R_t ~ Extreme Minimum Temperature	3	-0.6695
Adult	Precipitation	**R_t ~ Max Snow Water Equivalent***	3	**-2.7788**
		R_t ~ Mean Snow Water Equivalent*	3	**-2.7788**
		R_t ~ Accumulated Rainfall	3	-0.8856
		R_t ~ Julian Date of Snowmelt	3	-0.7088
	Temperature	**R_t ~ Extreme Maximum Temperature**	3	**-3.3465**
		R_t ~ Mean Maximum Temperature	3	-1.6039
		R_t ~ Mean Temperature	3	-1.4181
		R_t ~ Mean Minimum Temperature	3	-0.6702

Table 3: AIC comparison table for full generalized linear models including the best predictive weather and climate explanatory variables at each life stage (i.e., egg, larval-pupal, adult), their interactions, and density dependence on population change R_t. The best model for each life stage is listed in bold font.

Life Stage	Model	df	AIC
Egg	**R_t ~ logN_t + Mean Snow Water Equivalent + Mean Minimum Temperature**	5	**-7.7721**
	R_t ~ logN_t + Extreme Maximum Temperature + Mean Minimum Temperature	5	-7.2273
	R_t ~ logN_t + Mean Snow Water Equivalent + Extreme Maximum Temperature + Mean Minimum Temperature	6	-6.2467
	R_t ~ logN_t + Mean Snow Water Equivalent*Extreme Maximum Temperature + Mean Minimum Temperature	7	-4.8884
	R_t ~ logN_t + Extreme Maximum Temperature + Mean Snow Water Equivalent*Mean Minimum Temperature	7	-4.2761
	R_t ~ logN_t + Mean Snow Water Equivalent*Extreme Maximum Temperature + Mean Snow Water Equivalent*Mean Minimum Temperature	8	-3.3665
	R_t ~ logN_t	3	-3.0856
	R_t ~ 1	2	-2.6412
	R_t ~ logN_t + Mean Snow Water Equivalent + Extreme Maximum Temperature	5	-1.6514
	R_t ~ Mean Snow Water Equivalent	3	-1.3919
	R_t ~ Mean Minimum Temperature	3	-1.3306
	R_t ~ Extreme Maximum Temperature	3	-0.8014
	R_t ~ Extreme Maximum Temperature + Mean Minimum Temperature	4	0.1655
	R_t ~ Mean Snow Water Equivalent + Extreme Maximum Temperature	4	0.1886
	R_t ~ Extreme Maximum Temperature + Mean Snow Water Equivalent*Mean Minimum Temperature	6	0.2423
	R_t ~ Mean Snow Water Equivalent + Mean Minimum Temperature	4	0.3441
	R_t ~ Mean Snow Water Equivalent*Mean Minimum Temperature	5	1.3311
	R_t ~ Mean Snow Water Equivalent + Extreme Maximum Temperature + Mean Minimum Temperature	5	1.7033
	R_t ~ Mean Snow Water Equivalent*Extreme Maximum Temperature	5	2.0951
	R_t ~ Mean Snow Water Equivalent*Extreme Maximum Temperature + Mean Minimum Temperature	6	3.5334
	Likelihood Ratio Test of Null Model to Best Model F = 4.0271	3	0.1059
Larval-Pupal	**R_t ~ Extreme Maximum Temperature**	3	**-8.0743**
	R_t ~ Accumulated Rainfall + Extreme Maximum Temperature	4	-7.6590
	R_t ~ Accumulated Rainfall*Extreme Maximum Temperature	5	-6.4838
	R_t ~ logN_t + Extreme Maximum Temperature	4	-6.0747
	R_t ~ logN_t + Accumulated Rainfall + Extreme Maximum Temperature	5	-5.6658

	R_t ~ $logN_t$ + Accumulated Rainfall*Extreme Maximum Temperature	6	-4.7182
	R_t ~ $logN_t$	3	-3.0856
	R_t ~ 1	2	-2.6412
	R_t ~ $logN_t$ + Accumulated Rainfall	4	-1.8830
	R_t ~ Accumulated Rainfall	3	-1.3621
	Likelihood Ratio Test of Null Model to Best Model F = 9.1939	**1**	**0.02303**
Adult	**R_t ~ $logN_t$ + Extreme Minimum Temperature + Extreme Maximum Temperature**	5	**-4.7035**
	R_t ~ Maximum Snow Water Equivalent + Extreme Maximum Temperature	4	-4.3000
	R_t ~ Maximum Snow Water Equivalent*Extreme Maximum Temperature	4	-4.3000
	R_t ~ $logN_t$ + Maximum Snow Water Equivalent + Extreme Maximum Temperature	5	-3.8363
	R_t ~ Extreme Maximum Temperature	3	-3.3465
	R_t ~ $logN_t$	3	-3.0856
	R_t ~ Maximum Snow Water Equivalent	3	-2.7788
	R_t ~ $logN_t$ + Maximum Snow Water Equivalent + Extreme Minimum Temperature + Extreme Maximum Temperature	6	-2.7225
	R_t ~ $logN_t$ + Extreme Minimum Temperature + Maximum Snow Water Equivalent*Extreme Maximum Temperature	6	-2.7225
	R_t ~ $logN_t$ + Maximum Snow Water Equivalent*Extreme Minimum Temperature + Extreme Maximum Temperature	6	-2.7225
	R_t ~ $logN_t$ + Maximum Snow Water Equivalent*Extreme Minimum Temperature + Maximum Snow Water Equivalent*Extreme Maximum Temperature	6	-2.7225
	R_t ~ 1	2	-2.6412
	R_t ~ Extreme Minimum Temperature + Extreme Maximum Temperature	4	-2.4239
	R_t ~ Maximum Snow Water Equivalent + Extreme Minimum Temperature + Extreme Maximum Temperature	5	-2.3274
	R_t ~ Extreme Minimum Temperature + Maximum Snow Water Equivalent*Extreme Maximum Temperature	5	-2.3274
	R_t ~ Maximum Snow Water Equivalent*Extreme Minimum Temperature + Extreme Maximum Temperature	5	-2.3274
	R_t ~ Extreme Minimum Temperature	3	-1.0617
	R_t ~ Maximum Snow Water Equivalent + Extreme Minimum Temperature	4	-0.8080
	R_t ~ Maximum Snow Water Equivalent*Extreme Minimum Temperature	4	-0.8080
	R_t ~ $logN_t$ + Maximum Snow Water Equivalent + Extreme Minimum Temperature	5	0.4432
	Likelihood Ratio Test of Null Model to Best Model F = 2.3194	3	0.2170

Discussion

Life stage hypothesis modelling of a *Parnassius clodius* population from 2009 to 2018 identified extreme maximum temperatures during the larval-pupal life stage as a mechanism driving population dynamics in the next generation of the population (Figures 4 & 5). These findings do not support our original hypothesis, that weather conditions during the overwintering period (i.e., egg life stage) would have the greatest effect on population size. However, they clearly identify that the larval-pupal life stages are the most sensitive to changing weather and climate conditions for the montane species *Parnassius clodius*. When extreme maximum temperatures reach 22.5°C or above in May (i.e., larval-pupal life stage) there is a noted decline in *P. clodius* population growth rates, while additional unaccounted factors must be contributing to the deviation from this pattern observed in 2018 (Figure 5). This finding suggests that the predicted increasing frequency of short-term extreme weather events (van Aalst 2006), especially in May, and long-term climatic changes to increasing temperatures (Hostetler et al. 2021, IPCC 2021) could lead to continual declining *P. clodius* populations.

Related research investigating the response of population dynamics of the related non-migratory montane univoltine butterfly, *Parnassius smintheus*, identified direct and indirect effects of weather and climate on population growth rates. A field study showed that a quadratic interaction between extreme minimum temperature and absence of snow cover had the greatest *direct* negative effect on *P. smintheus* overwintering egg survival, with temperatures being too hot or cold and little to no snow negatively affecting population growth (Roland and Matter, 2016). This finding supported our hypothesis that weather and climate during the overwintering period would primarily affect population growth, however, our findings for *P. clodius* indicate

that weather conditions during the larval-pupal life stages have the greatest effect. In a rearing experiment using *P. smintheus*, elevated temperatures during larval development had a *direct* negative effect on survival and population growth due to early larval emergence (Matter et al. 2011). The rearing experiment detected similar findings to ours, with elevated temperatures during larval development having a negative effect on population growth. However, our research did not allow us to distinguish direct from indirect effects such as early host plant senescence on population dynamics. Increasing the years of observation and data on host plant phenology would aid in detecting a definitive relationship for *P. clodius*.

From the perspective of developing a more generalized understanding of montane insect responses to climatic variation, we can summarize the responses of these two related butterfly species by saying that the indicator life stage of *P. smintheus* is the overwintering egg, whereas the indicator life stage of *P. clodius* is the larval-pupal life stages in spring. Extreme summer temperatures (i.e., hot and cold) are speculated to have *direct* negative effects on *P. smintheus* adult survival (Matter et al., 2011). These results are inconsistent with our findings, suggesting that population dynamics in response to weather conditions and climate change vulnerability may not be consistent across a genus. In fact, *P. clodius* and *P. smintheus* population dynamics responded to different weather and climate variables at different life stages.

From the perspective of plant-insect interactions under varying climate, altered temperature and precipitation affecting growth and survival of host and nectar plant species limiting larvae and adult resources was identified as a negative *indirect* effect on *Parnassius smintheus* presence, distribution, and genetic structure (Matter et al. 2011, Filazzola et al. 2020). Similarly, research evaluating the reproductive success of the threatened univoltine butterfly,

Euphydryas editha bayensis, found that later adult emergence and early host plant senescence had *direct* and *indirect* negative effects on female reproductive success (Cushman et al., 1994; USFWS, 2023). Prediapause (larval life stage) starvation resulting from early host plant senescence was identified as the leading cause of *E. editha bayensis* mortality (Cushman et al., 1994; USFWS, 2023). The *indirect effects* of early host plant senescence on *Euphydryas editha bayensis* population dynamics are applicable to our findings, given that the timing of host plant emergence, senescence, and survival dictated larval and pupal survival and were reflected in the population dynamics of this univoltine butterfly species. Therefore, conservation of univoltine butterfly species should be directed towards evaluating the distribution, phenology, fecundity, and survival of a butterfly species at each life stage in addition to its nectar and host plant species (Demarse et al. 2023), especially when the butterfly is specific to one host plant species.

The next steps to foster additional insights on *P. clodius* growth rates would include evaluating the distribution and phenology of its host plant, *Dicentra uniflora*, an early-spring ephemeral forb relative to climatic data that we used in assessing *P. clodius* population dynamics. The life history of *D. uniflora* has been examined in the southern extent of the Cascade Range in California, U.S.A., but little is known about its phenology in the Rocky Mountain Region in the Western U.S.A. (Schlising and Mackey 2020, Crees and Debinski 2021). *Dicentra uniflora* emergence and senescence has been observed to vary by up to two weeks at the same elevation depending on sun exposure within the region where *P. clodius* was evaluated in this research (Crees and Debinski 2021). No research has been conducted evaluating the temperature thresholds of *D. uniflora* (i.e., timing of emergence and senescence in response to temperature). This has direct implications for the research presented here as we speculate that increasing

temperatures during *D. uniflora*'s flowering duration, and *P. clodius*'s larvae life stage, could lead to early senescence thereby initiating larvae starvation and declining *P. clodius* larval-pupal survival. Ultimately, increasing our understanding of *D. uniflora*'s phenology and sensitivity to climatic variation will aid in conserving both *D. uniflora* plants and *P. clodius* butterflies, and both could serve as bioindicators of weather patterns or climatic change in montane ecosystems.

Host plant life history and phenology affect the population change of numerous butterfly species, including *P. clodius* and *P. smintheus*. *Parnassius smintheus*' host plant, *Sedum lanceolatum*, is a succulent species (Roland and Matter 2016) persisting throughout the summer months withstanding moderate drought and heat, while *Dicentra uniflora*, *P. clodius*' host plant, is a spring ephemeral and much more vulnerable to drought and heat. The differences between these host plant's life histories influence their responses to weather conditions and phenology ultimately affecting those species that interact and depend on them. For instance, the research presented here identified vulnerability using *P. clodius* population growth rates during the larval-pupal life stage in response to extreme heat. This could be the *direct* result of larvae and/or pupae being vulnerable to heat or the *indirect* result of host plant vulnerability leading to early senescence and larval starvation. We were unable to clearly identify at this time which metric was responding to extreme heat driving the declining population growth trends, however, we suspect an *indirect* effect of host plant vulnerability due to *D. uniflora*'s life history characteristics. Understanding host plant life histories and potential vulnerabilities may aid in identifying indicator life stages for butterfly species.

Findings from this study and previous research demonstrate the value in assessing butterfly responses to short-term weather variation or long-term climatic changes *at each life*

stage in order to protect and conserve a species and its interactions with other organisms (Radchuk et al. 2013, Halsch et al. 2021, Buckley 2022). This research serves as a model for understanding more threatened *Parnassius* species and other butterfly species. Life stage hypothesis modelling would aid in identifying vulnerable life stages of threatened butterfly species, helping guide conservation efforts to preserve populations. Population predictions and conservation efforts based on a single life stage would likely incorrectly indicate that a species is not directly at risk of global climate change (Radchuk et al. 2013). Therefore, evaluation of all life stages is warranted to correctly identify vulnerability and an indicator life stage. Further, an understanding of host plant life histories along with data on host plant phenology in response to weather variations would provide guidance to conservation efforts. Additionally, it is pertinent to evaluate species responses during fall and spring when extreme fluctuations in weather occur most frequently and have been observed to have the greatest impact on species, especially montane butterflies (Matter et al. 2011). The research presented here, using life stage hypothesis modelling, determined extreme maximum temperatures during the larval-pupal life stages of *Parnassius clodius* led to a decline in the next generation (Figure 5). As exemplified in this research, evaluating an insect species' response to external stressors by life stage reveals at which life stage conservation efforts should be focused in order to efficiently protect that species.

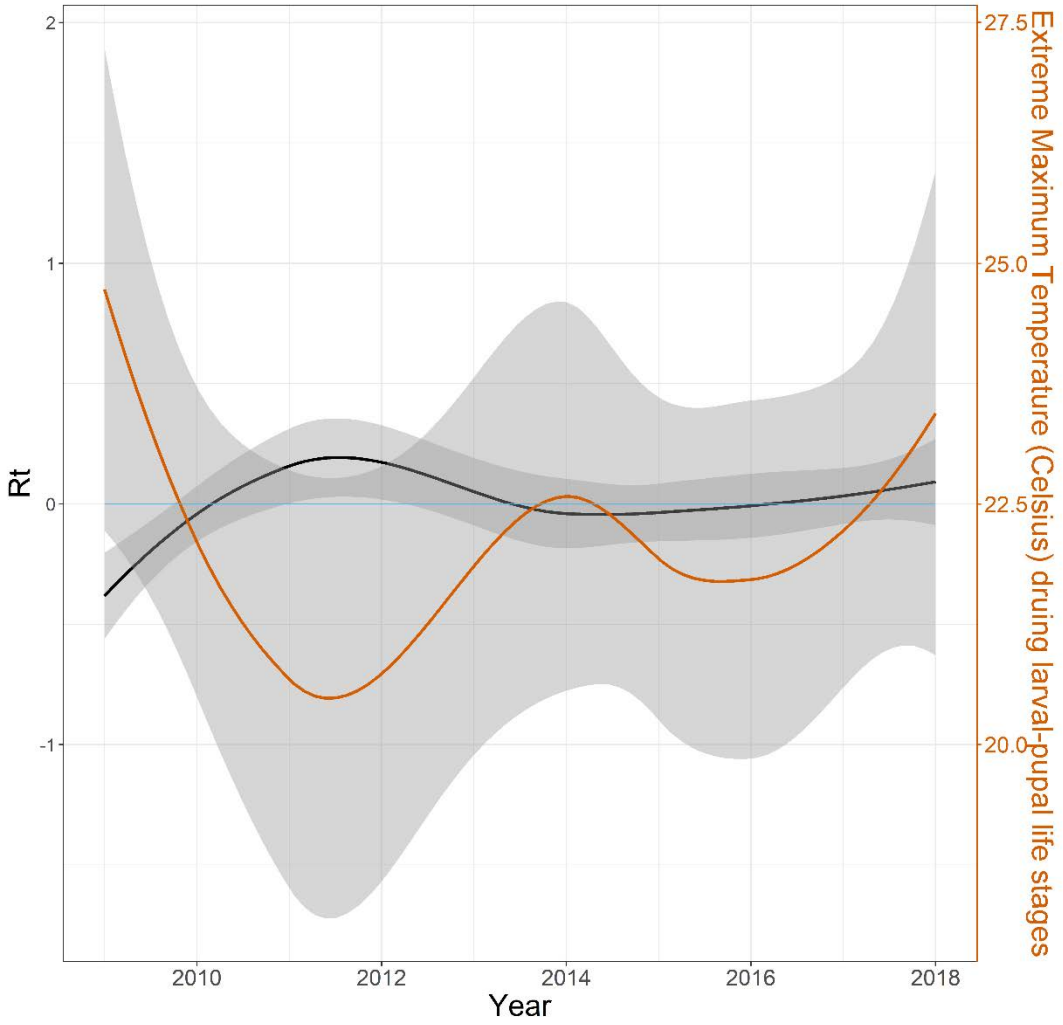


Figure 5: *Parnassius clodius* population change (R_t) from 2009 to 2018 relative to the extreme maximum air temperature of its environment during the larva-pupa life stages. Note: surveys were not carried out in 2012 and 2013.

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CHAPTER THREE

EARLIER SPRING SNOWMELT DRIVES ARROWLEAF BALSAMROOT PHENOLOGY IN MONTANE MEADOWS

Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 2

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Contributions: consulted on data analysis methodology and contributed to drafting manuscript for publication

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Abstract

Climate change is shifting phenology globally, altering when and how species respond to environmental cues such as temperature and the timing of snowmelt. These shifts may result in phenological mismatches among interacting species, creating cascading effects on community and ecosystem dynamics. Using passive warming structures and snow removal, we examined how experimentally increased temperatures, earlier spring snowmelt, and the poorly understood interaction between warming and earlier spring snowmelt affected flower onset, flowering duration, and maximum floral display of the spring flowering montane species, Arrowleaf Balsamroot (*Balsamorhiza sagittata*), over a seven-year period. Additionally, potential cumulative effects of treatments were evaluated over the study duration. The combination of heating with snow removal led to earlier flower onset, extended flowering duration, and increased maximum floral display. While there was year-to-year variation in floral phenology, the effect of heating with snow removal on earlier onset and maximum floral display strengthened over time. This suggests that short-term studies likely underestimate the potential for climate change to influence phenological plant traits. Overall, this research indicates that *Balsamorhiza sagittata*'s flowering onset responded more strongly to snow removal than to heating, but the combination of heating with snow removal allowed plants to bloom earlier, longer, and more profusely, providing more pollinator resources in spring. If warming and early snowmelt cause similar responses in other plant species, these patterns could mitigate phenological mismatches with pollinators by providing a wider window of time for interaction and resiliency in the face of change. This example demonstrates that a detailed understanding of how spring-flowering plants respond to specific aspects of predicted climatic scenarios will

improve our understanding of the effects of climate change on native plant-pollinator interactions in montane ecosystems. Studies like this help elucidate long-term physiological effects of climate-induced stressors on plant phenology in long-lived forbs.

Introduction

Climate change is a growing issue, leading to species declines and influencing species interactions globally (Valiente-Banuet et al. 2015). Environmental cues, like temperature and the timing of spring snowmelt, initiate species behaviors and physiology patterns. Alteration of the timing of environmental cues associated with climate change can induce deviations in a species' typical lifecycle pattern (i.e., shift in phenology) and affect onset and duration of biological events for both flora and fauna (Walther et al. 2002, Parmesan and Yohe 2003). Phenological shifts may have detrimental effects on plant communities and those species with which they interact. Even minute shifts in the timing, magnitude, and spatial extent of these environmental cues can have cascading effects on community dynamics via species abundance and distribution shifts (Parmesan and Yohe 2003) and have the potential to lead to phenological mismatch between species (Visser and Both 2005). Phenological shifts and mismatches can impact species fitness, abundance, and persistence in a community, creating lasting effects on overall ecosystem structure and function (Burkle and Alarcon 2011).

Previous studies have examined the separate effects of warming and snowmelt on flowering phenology and flower production in montane systems. Earlier flowering has been documented under warmer temperatures (Arft et al. 1999, Fitter and Fitter 2002, Kehrberger and Holzschuh 2019, Jabis et al. 2020) and advanced spring snowmelt, with spring flowering plants being particularly sensitive to the timing of snowmelt (Steltzer et al. 2009, Wipf 2010, Gezon et

al. 2016, Wadgymar et al. 2018). Increased flower production has been observed with experimental warming in tundra ecosystems spanning the Arctic (Arft et al. 1999) and with experimentally advanced snowmelt in Colorado subalpine meadows (Gezon et al. 2016). Conversely, a study conducted on the Tibetan plateau reported decreased flower production with simulated warming (Liu et al. 2012). A yearlong greenhouse warming experiment resulted in shortened flowering duration due to earlier plant senescence following typical flowering onset (Nagahama et al. 2018). These studies highlight that montane and alpine plant species experience altered phenology in response to warming and/or early snowmelt.

A few studies have observationally or experimentally examined the separate and combined effects of warming and advanced snowmelt on plant individuals in montane environments over a period of time longer than four years. These studies in the Rocky Mountains of Colorado found earlier flowering onset with warming and advanced snowmelt (Dunne et al. 2003, Wadgymar et al. 2018, Jabis et al. 2020), increased floral displays with earlier snowmelt (Inouye 2008), and extended flowering duration in response to earlier melt (Price and Waser 1998, Dunne et al. 2003, Jabis et al. 2020), indicating that the phenologies of plant species in this montane ecosystem are tracking climate change. By contrast, Inouye (2008) found reduced floral abundance for three early spring flowering perennials (i.e., *Delphinium barbeyi*, *Erigeron speciosus*, and *Helianthella quinquenervis*) associated with earlier snowmelt in a 32-year observational study. The reduction in floral abundance was due to convective frost events (i.e., cold air masses) with trending lower air temperatures after snowmelt which damaged flower buds (Inouye 2008). In the same montane ecosystem, extended flowering duration, especially for spring-flowering species, was observed over a four-year period in response to a naturally-

occurring and experimentally-manipulated earlier snowmelt and warmer spring soil temperatures (Dunne et al. 2003). Thus, phenological responses to temperature-related climate change were detected within a four-year study period, which could lead to longer term asynchronies in interspecific interactions. For the early flowering perennial, *Delphinium nuttallianum*, experimental warming reduced floral abundance (i.e., the number of quadrats in which *D. nuttallianum* was present) and the number of flowers per plant over a six year period, suggesting that global warming could affect the fecundity of montane flowering species and floral resources available for pollinators (Saavedra et al. 2003). Jabis et al. (2020) detected earlier flower onset of forbs in response to experimental heating and heating-induced earlier snowmelt, with the timing of snowmelt date driving the pattern, especially for early-season species. This four-year study uncovered that forbs respond more strongly to changes in temperature that are beneficial for reproduction and local plant-pollinator communities.

Arrowleaf Balsamroot (*Balsamorhiza sagittata*) is a vital early-spring floral resource for pollinators in montane regions just after snowmelt. In addition to its role as a nectar source for generalist pollinators, *B. sagittata* is a valuable source of nectar for the butterfly *Parnassius clodius* (Sherwood et al. 2017), a spring butterfly species know to interact with *B. sagittata*. Additionally, it serves as a critical nectar source for some specialized bee species in the genus *Osmia*, which forage exclusively on *Balsamorhiza* and *Wyethia* species (Cane 2011). Therefore, *B. sagittata*'s demography, phenology, and flower production are important for the stability of pollinator community dynamics in montane regions in parts of the western United States in early spring. We experimentally investigated the effects of warming, advanced snowmelt, and their less examined interactive effects in a factorial design on phenology and floral production of the

perennial plant *B. sagittata* in a montane meadow in Wyoming, USA over seven years. Specifically, we investigated treatment effects on flower onset, flowering duration and maximum floral display, the cumulative treatment effects over time, and the correlations among plant responses to treatments to understand how flower onset, flowering duration, and maximum floral display are associated. The following expectations and predictions are based on previous research. We expected earlier flowering onset with all non-control treatments with the earliest onset occurring in the heating and snow removal treatment in response to earlier light access and warmer spring temperatures (Arft et al. 1999, Fitter and Fitter 2002, Steltzer et al. 2009, Gezon et al. 2016, Wadgymar et al. 2018, Kehrberger and Holzschuh 2019, Jabis et al. 2020). We predicted extended flowering durations with snow removal in response to earlier onset and access to light (Price and Waser 1998, Dunne et al. 2003), but no change with heating and snow removal, as the two treatments could counteract each other (Nagahama et al. 2018). We expected increased maximum floral display with snow removal in response to earlier onset and extended flowering duration which allows plants to flower longer and potentially produce more flowers (Gezon et al. 2016). However, we predicted a decrease in maximum floral display in response to the combination of heating and snow removal due to temperature and soil moisture stress (Saavedra et al. 2003). Further, we expected that the effects of each treatment on *B. sagittata* flowering onset, flowering duration, and maximum floral display would strengthen over the course of the study. All above hypotheses are listed in Table 4. This study contributes unique insights to the potential for advanced snowmelt to interact with warming to influence flowering phenology and the degree to which these treatment effects strengthen over time.

Table 4: Hypotheses about how *B. sagittata* flower onset, flowering duration, maximum floral display may respond to decreased spring snowmelt via removal, warming via passive heating, and warming with decreased spring snowpack.

Predictions	**Flower Onset**	**Flowering Duration**	**Maximum Floral Display**
Decreased spring snowpack via snow removal (SR)	*Earlier* in response to earlier access to light	*Extended* in response to earlier flower onset with earlier light access	*Increase* due to earlier flowering onset and extended flowering duration
Warming via passive heating (H)	*Earlier* in response to gradually increasing winter and spring temperatures	*Shortened* due to asymmetrical shift in flowering onset and senescence	*Decrease* in response to higher temperatures and decreased soil moisture causing stress
Warming with snow removal (H+SR)	*Earlier* due to the interactive effect of increasing temperatures and access to light	*Similar to control* in response to interactive effect of warming and snow removal canceling each other out	*Decrease* in response to higher temperatures and decreased soil moisture causing stress
SR * Year, H * Year, and H+SR * Year	The effect of treatment will *strengthen* over the seven-year study	The effect of treatment will *strengthen* over the seven-year study	The effect of treatment will *strengthen* over the seven-year study

Methods and Materials

Study Site

This study was conducted from 2013-2019 in a flat montane meadow near Pilgrim Creek at an elevation of 2100 m in Grand Teton National Park, Wyoming, United States (43.914977°; -110.578072°). Meadow vegetation composition consists of various sagebrush species (*Artemisia* spp.) and herbaceous flowering plants and grasses, but is dominated by bare ground (about 50% cover). The soil surface is highly permeable, consisting of gravel glacial outwash deposited by the Pinedale glaciers (Martin 2008). The regional climate is characterized by a mean annual precipitation of 60 cm with most of that falling as snow (Western Regional Climate Center, Moran, WY). The mean snow depth (i.e., all season snow accumulation) is 6.4 m, with snow cover beginning in October and ending in May (NRCS SNOTEL: Base Camp). Mean monthly temperatures ranged from -32 to 21 °C from 1988 to 2019; December and January are the coldest

months and July and August are the warmest (NRCS SNOTEL: Base Camp). The growing season typically begins in mid-May with snowmelt and extends to the end of August or early September (Debinski et al. 2000).

Experimental Methods and Design

Twelve 2.4 m^2 (8 ft x 8 ft) plots were established on existing native plant communities in this meadow in 2010 and monitored annually. Across the 12 plots, the number of *Balsamorhiza sagittata* plants in a plot ranged from six to 26 plants across the study duration. Heat and snow removal were manipulated in a factorial design to create four treatments (N = 3 replicates per treatment): decreased spring snowpack via snow removal (SR: not heated, snow removal), warming via passive heating (H: heated, no removal), heating and snow removal (H+SR: heated, snow removal), and control (C: not heated, no removal) (Sherwood et al. 2017). Within each of the three blocks (East, Central, and West), treatments were randomly assigned to plots, with one treatment replicate per block (Figure A1). Treatments assigned to plots remained the same for the duration of the study. Plots were regularly spaced at 4.9 m (16 ft) apart from one another. Decreased spring snowpack, simulating early snowmelt, was achieved by manually removing snow from designated plots in late April or early May prior to the growing season, leaving about 2 cm of snow to prevent vegetation damage. Snow removal was accomplished in all except for two years (2015 and 2016) when snowpack was too low to allow for removal. Snow depths removed ranged from 55 cm to 80 cm for the years when snow was removed. Heating was accomplished by constructing a 2.4 m^2 (8 ft x 8 ft) passive warming structure. The top of the structure was 59 cm from the ground as to not impede plant growth. During 2010-2015, these structures were made of wood and plexiglass (Sherwood et al. 2017). From 2016-2018, the

structures were made of wood with a greenhouse plastic roof with perforations to allow precipitation to fall through. In 2019, these structures were made of PVC pipe with a greenhouse plastic roof with small perforations. All warming structures enhanced the downward reflectance of infrared radiation on the ground and increased the daily minimum temperatures of plant and soil surfaces by 2 °C (Sherwood et al. 2017). Mean surface temperatures in Western North America are projected to increase by 1-7 °C by 2100 (IPCC 2014). The passive warming structures used here increase surface temperature on the low end of regional climate projections, therefore resulting responses to warming are a conservative response. Passive warming structures were installed on designated plots at the time of snow removal (i.e., late April, early May) prior to the growing season, and remained in place until late September or October after the growing season was completed (Sherwood et al. 2017). Soil and surface temperatures for each plot were recorded every hour throughout the growing season using a soil temperature probe buried 25 cm below the surface and HOBO loggers placed on the soil surface in the center of the plot. Ambient air temperature was recorded for the set of plots every hour throughout the growing season using an air sensor placed one meter above the soil surface.

All individual *Balsamorhiza sagittata* plants 10 cm or taller growing within each of the 12 plots were measured every three days from emergence (mid-May) to senescence (mid-July). A *B. sagittata* individual takes three to five years to flower and remains reproductively active for up to 40 years (Tilley et al. 2012, Gucker and Shaw 2018). Individual plants were numbered and monitored across years using plot maps and metal identification tags (Figure A2). Measurements, which occurred ca. every 3 days during the growing season, included whether the individual was dead or alive and, if alive, flower phenology (i.e., buds, fully flowering, senescing, and

senesced). Flowering heads were considered fully open once at least one disk flower opened, senescing once ray flowers withered but open disk flowers remained, and senesced once disk flowers withered and seed maturity was observed. We distinguished among these stages because fully open and senescing flowers are the only stages that provide nectar resources for pollinators (Potts et al. 2003) that feed on *B. sagittata* in montane meadows, and we wanted to quantify this window of time for each treatment. Within an individual *B. sagittata* plant, buds and flowers often co-occur with blooming occurring sequentially, where the bloom and senescence of an individual flower is followed by another, across primarily solitary floral heads (Tilley et al. 2012, personal observations).

Statistical Analysis

Statistical analyses were performed to determine whether flowering onset (i.e., date of first flower), flowering duration, or maximum floral display of individual plants differed among treatments across the seven-year study and whether the effect of treatment grew stronger over time. A 4-level categorical variable that incorporates the main effects of heating and snow removal along with their interaction was created using the *interaction* function in R. The four levels are described in the experimental methods and design. This variable was included as a predictor variable in all models and statistical tests. Individual plants that failed to produce flowers because they had not yet reached flowering or reproductive maturity were excluded from these analyses. Those individuals were removed in order to avoid the potential for plant age to skew maximum floral display estimates unrelated to treatment effect. A total of 88 individual plants that were alive between 2013-2019 and flowered at least once were included in these analyses. All analyses were evaluated at a significance level of 0.05, where values <0.05 indicate

significance, 0.05 to 0.1 indicate marginal significance, and >0.1 indicates no significance. Missing values were removed prior to model construction. For example, plants that produced a flower one year but didn't produce in subsequent years were removed from the analyses, while plants that produced a flower one year but skipped a year of flowering and continued to flower in a subsequent year(s) remained in the analyses. Skipped years were represented with a "0" value. Mixed effects models were used to test the effects of treatment on response variables, and included individual plant identity nested within plot as random effects to account for multiple measurements of each individual across plots over the seven-year duration of this study. When testing the effect of treatment, year was included as a crossed additive random effect in each mixed effects model to control for random year-to-year variation around the patterns of the treatments. This allowed us to assess how treatment affected each variable while accounting for year-to-year differences in weather and other conditions. Linear mixed effects models with a Tukey contrast were used to test for multiple comparisons.

To test whether treatment effects on flower onset, flowering duration, and maximum floral display took time to develop (cumulative effects of treatment) or grew stronger over time, year was included as an interaction with treatment in each mixed effects model. Initial evidence of an interaction between treatment and year was supported by an F-test prior to further analysis and interpretation. Pairwise comparisons of treatment-year combinations are not shown because absolute values of treatment effects depended on year.

Flowering onset (i.e., date of first flower) was defined as the date on which an individual plant was observed to have at least one fully opened flower. Two linear mixed effect models (described above) were used to identify whether the effects of treatment on flower onset varied

across the seven-year study and whether the effects of treatment on flower onset grew stronger over the seven-year period.

Duration of flowering was defined as the number of days between observation of an individual's first fully open flower and its last senescing flower in a given growing season. In some instances, an individual was observed to be flowering on only one date in a particular year, prohibiting the estimation of a flowering duration for that individual in that year. Such individuals were excluded from these analyses. Linear mixed effects models were fit to identify whether the effects of treatment on flowering duration varied across the seven-year study and whether the effects of treatment on flowering duration grew stronger over the seven-year period.

Maximum floral display (i.e., maximum number of concurrent flowers) was calculated by totaling the number of fully flowering, senescing, and senesced flowers of an individual at each visit to identify the maximum number of flowers present concurrently on an individual in a given year. Buds were not included in these counts because the number of flowers produced was intended as a proxy for the quantity of available nectar, and buds that failed to flower provided no nectar. A sum of all flowers produced by an individual over the growing season would not be reliable due to the potential for the same flower to be counted multiple times on successive visits. Using the maximum number of concurrent flowers eliminates the possibility of recounting the same flowers, providing a conservative estimate for the number of flowers produced by an individual over each growing season. Additionally, disintegrated and fallen senesced flowers were excluded when using the maximum number of concurrent flowers metric. Generalized linear mixed effects models with a negative binomial distribution to account for overdispersion were fit using these estimates to determine whether the effects of treatment on maximum floral

display varied across the seven-year study duration and whether the effects of treatment on maximum floral display grew stronger over the seven-year period.

The raw data over the seven-year study were visually examined to investigate the associations between flower onset, flowering duration, and maximum floral display of *Balsamorhiza sagittata* within each treatment to explore potential relationships between these response variables. Models prove difficult to use in this instance due to the multicollinearity of using response variables as predictor variables for other response variables in the same study. Here we chose to present graphed relationships using raw data collected in this study and their associated correlation values to draw attention to possible relationships. The correlation coefficient was calculated using the *cor.test* function to evaluate the association between these variables for each treatment. All raw observations in each treatment across the seven-year study were pooled.

Mixed effects models were constructed using the *lmer* and *glmer.nb* functions in the LME4 package (Bates et al. 2015) with the LMERTEST package (Kuznetsova et al. 2017) to obtain p-values in R and the *glmmadmb* function in the GLMMADMB package (Fournier et al. 2012; Skaug et al. 2016). Estimated marginal means pairwise comparisons were completed using the *emmeans* function in the EMMEANS package (Lenth 2020) in R to create figures with letters identifying significance between treatments. Multiple comparisons of means using a Tukey contrast for each mixed effects model was conducted using the *glht* function in the MULTCOMP package (Hothorn et al. 2008) in R to determine relative effects of treatment. Figures were created using the GGPLOT2 package (Wickham 2016) in R (R Core Team 2020).

Results

Flower Onset

Heating mediated the influence of snow removal on flowering onset (interactive effect between heating and snow removal; Table 5). There were also main effects of snow removal and heating (Table 5). Snow removal (SR) and heating with snow removal (H+SR) advanced flowering onset by 4.0 and 3.8 days respectively, while heating alone (H) advanced onset by 1.9 days compared to the control (C) treatment, though the difference in heated effects was not significant (Figure 6). The multiple comparison of means with a Tukey contrast showed a significant interaction between heating and snow removal on flower onset (Table 5), but snow removal appears to have the strongest effect on flower onset (Figure 6). The treatment effects of heating and heating with snow removal strengthened over the seven-year study duration significantly (Table 5). For instance, *B. sagittata* flowering onset occurred earlier over time with snow removal (SR) and heating with snow removal (H+SR) and occurred later over time in the heating (H) and in the control (C) treatments (Table 6, Figure 7).

Flowering Duration

Both heating and snow removal individually extended flowering duration, and heating with snow removal (H+SR) had a significant effect, extending flowering duration by 5.0 days compared to the control (C) treatment. Flowering duration was 3.6 days longer in the snow removal treatment (SR) and 3.4 days longer in the heated (H) treatment compared to plants in the control (C) treatment (Figure 6). These increases in *B. sagittata* flowering duration were captured in the multiple comparison of means with a Tukey contrast across all treatments (Table 5). For one of the treatments (heating, H), there was a discrepancy in the effects of heating on flowering

duration between the two statistical tests that we used: while heating did not affect flowering duration according to estimated marginal means (Figure 6), heating did significantly extend flowering duration according to the linear mixed effects model with Tukey test (Table 5). The latter test is more powerful because it tests for multiple comparisons (Meier 2021), and, we summarize our results based on Table 5. There was no evidence that the magnitude of treatment effects on *B. sagittata* flowering duration changed over time (Table 6, Figure 7). The slopes of the relationships between flowering duration and year for all treatments (i.e., control (C), heating (H), snow removal (SR), and heating with snow removal (H+SR) are nearly zero, indicating that the strength of cumulative treatment effects was relatively consistent across the seven-year study (Figure 7).

Maximum Floral Display (i.e., Maximum Number of Concurrent Flowers)

Heating mediated the influence of snow removal on *B. sagittata*'s maximum floral display (interactive effect between heating and snow removal; Table 5). Maximum floral display was higher in the heating with snow removal treatment by 1.2 flowers compared to the control treatment (Figure 6). The treatment effect of heating with snow removal (H+SR) strengthened over the seven-year study significantly, with a slight increase in *B. sagittata* maximum floral display (Table 6, Figure 7). Treatment effects on maximum floral display were consistent across all years: plants in the heating with snow removal (H+SR) treatment had the most flowers while those in the control (C) treatment had the fewest, regardless of year (Figure 7).

Relationships Between Flower Onset, Flowering Duration, and Maximum Floral Display

Flower onset and duration were negatively associated with each other across all treatments, indicating that *B. sagittata* plants that began flowering earlier also bloomed for a longer period of time. Within treatments, heat with snow removal (correlation = -0.55, t = -5.66, df = 75, p-value < 0.0001) and snow removal (correlation = -0.53, t = -5.92, df = 88, p-value < 0.0001) exhibited the strongest negative correlation, while the control (correlation = -0.38, t = -3.76, df = 82, p-value = 0.0003) and heated (correlation = -0.33, t = -3.40, df = 96, p-value = 0.0010) treatments had the weakest negative correlation (Figure 8).

Flowering duration and maximum floral display were positively associated across all treatments, with plants that bloomed for a longer duration also having a larger maximum floral display. Within treatments, snow removal (correlation = 0.52, t = 5.67, df = 88, p-value < 0.0001) and the control (correlation = 0.32, t = 3.06, df = 82, p-value = 0.0030) indicated the strongest positive correlation, while heated (correlation = 0.18, t = 1.82, df = 96, p-value = 0.0718) and heat with snow removal (correlation = 0.17, t = 1.49, df = 75, p-value = 0.1404) had the weakest positive correlations with no significant support (Figure 8).

There was a negative relationship between flower onset and maximum floral display across all treatments, with *B. sagittata* plants that began flowering later also having a smaller maximum floral display. Within treatments, the control (correlation = -0.27, t = -2.54, df = 82, p-value = 0.0128) and heated (correlation = -0.24, t = -2.40, df = 96, p-value = 0.0183) treatments exhibited the strongest positive correlation, while snow removal (correlation = -0.21, t = -2.00, df = 88, p-value = 0.0512) and heated with snow removal (correlation = -0.09, t = -0.79, df = 75, p-value = 0.4329) had the weakest negative correlations with no significant support (Figure 8).

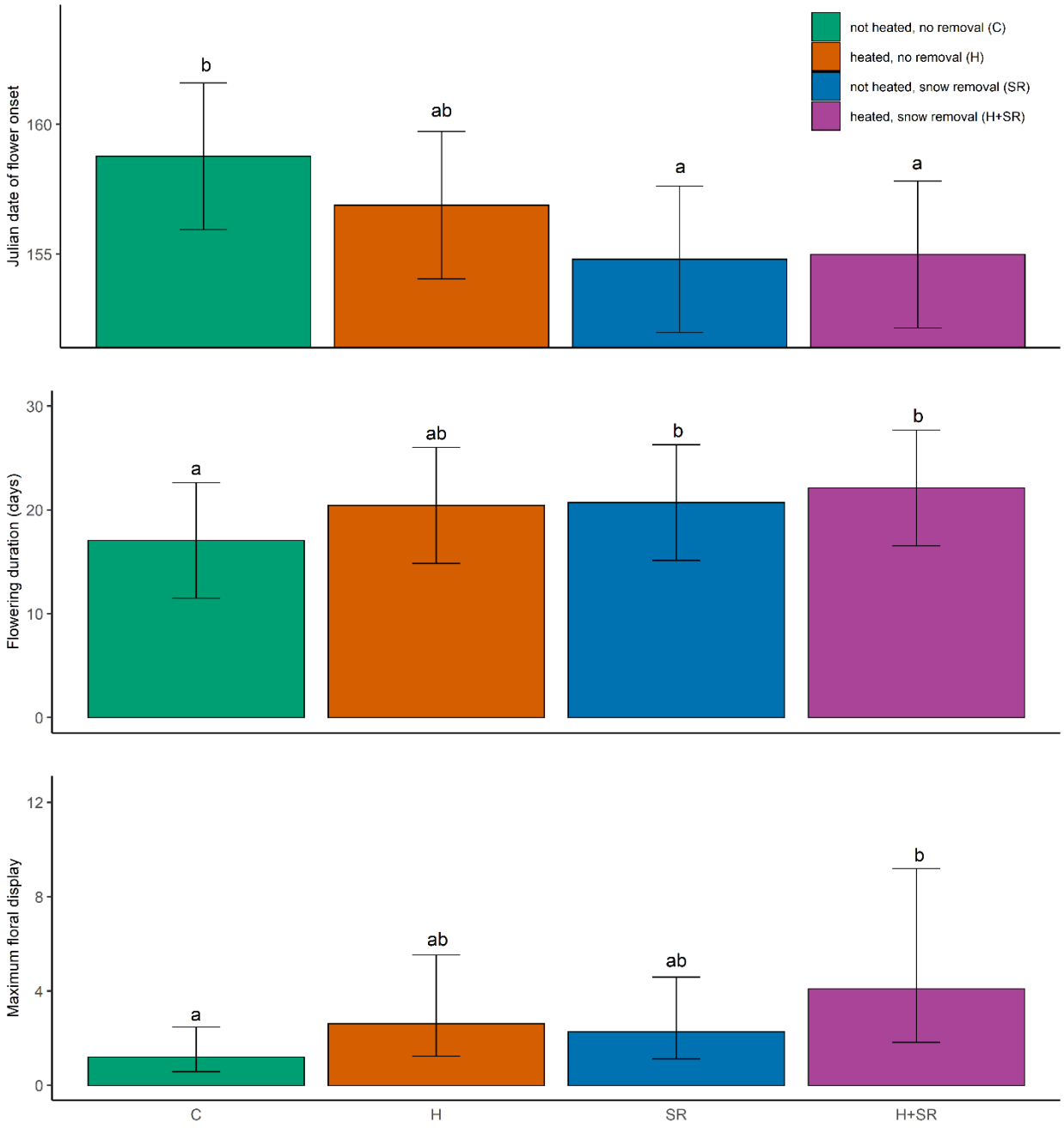


Figure 6: The effect of treatment on the least squares means flower onset date, flowering duration, and maximum floral display. Treatments included: decreased spring snowpack via snow removal (SR: not heated, snow removal), warming via passive heating (H: heated, no removal), decreased spring snowpack and warming (H+SR: heated, snow removal), and control (C: not heated, no removal). Estimated marginal means pairwise comparison are indicated by letters where the same letter indicates overlap and different letters indicate variation. The error bars represent the upper and lower 95% confidence levels.

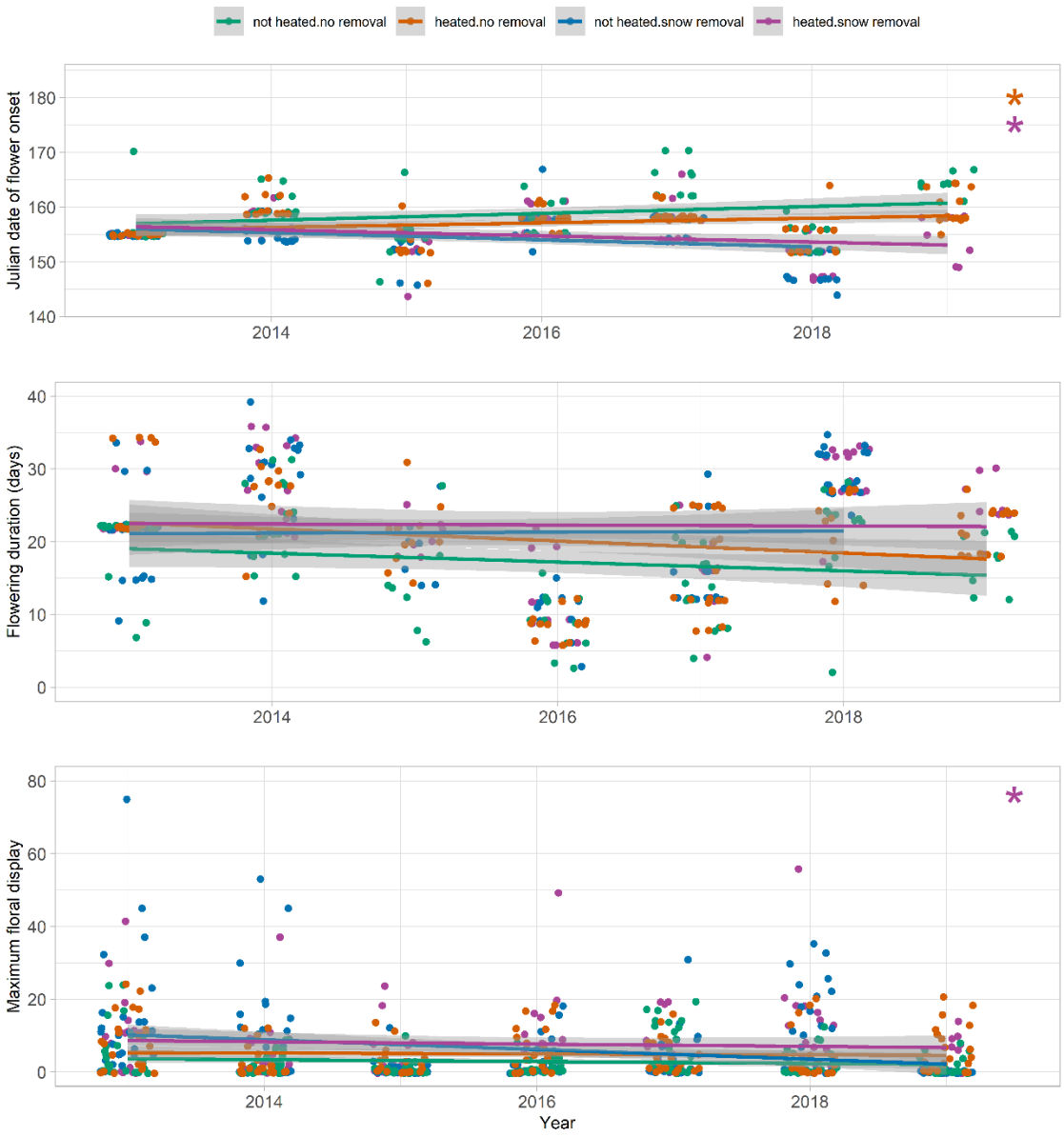


Figure 7: Treatment effects over time on flower onset, flowering duration, and maximum floral display. Treatments included: decreased spring snowpack via snow removal (SR: not heated, snow removal), warming via passive heating (H: heated, no removal), decreased spring snowpack and warming (H+SR: heated, snow removal), and control (C: not heated, no removal). Figure displays results of treatment interactions with year. Asterisks indicate a significant treatment × year interaction (indicated by treatment color) compared to the control on the response variable (i.e., flowering onset, flowering duration, and maximum floral display). The normal confidence intervals (indicated by the gray shaded areas along each line) were constructed using generalized linear smoothing to observe linear change in each treatment over time. Flowering onset did not occur in snow removal plots in 2019, therefore, there is no data available for that treatment and year.

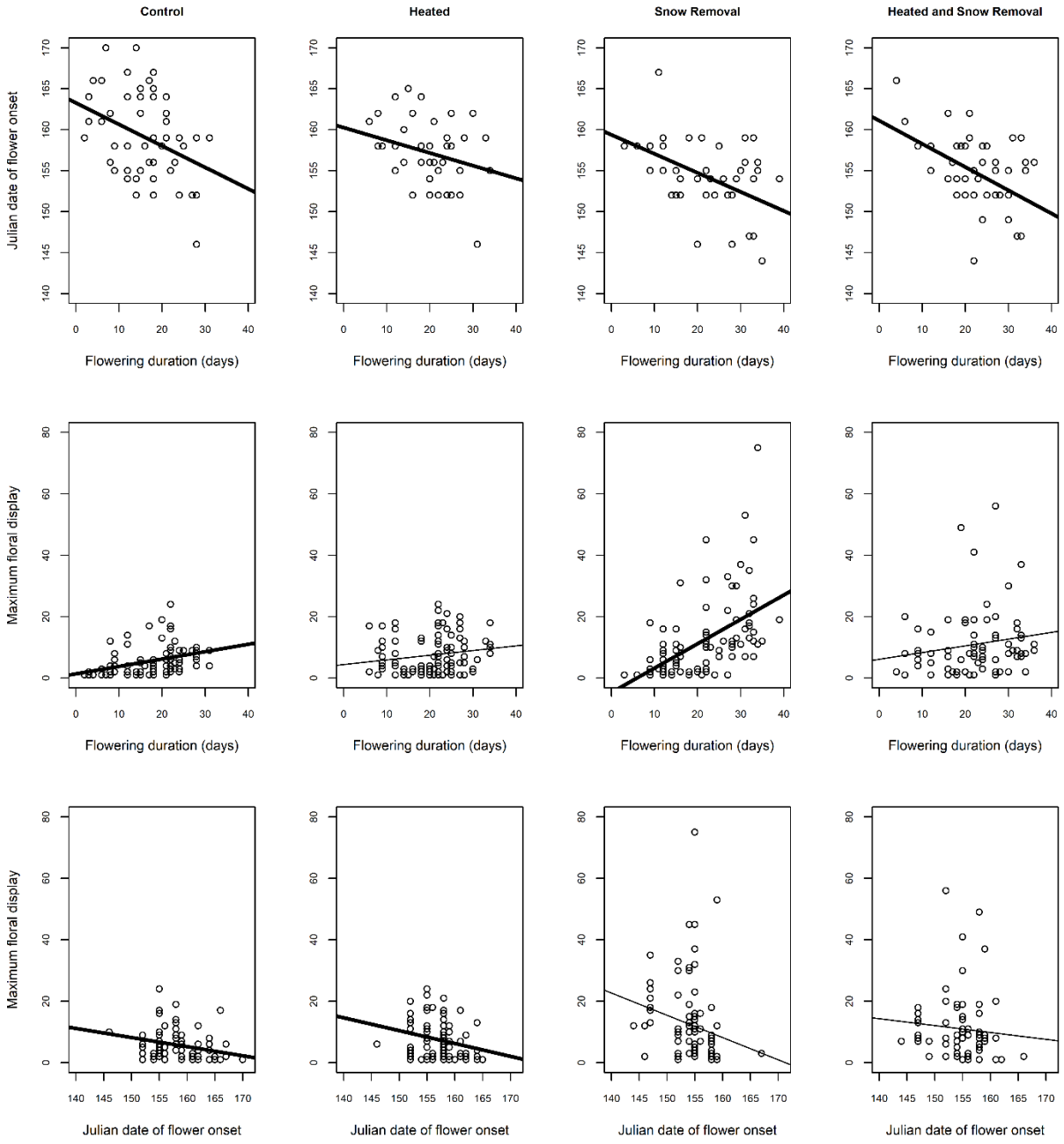


Figure 8: The relationships between the raw values for plant individuals of *Balsamorhiza sagittata* flower onset and flowering duration, flowering duration and maximum floral display, and flower onset and maximum floral display within each treatment across the seven-year study. Treatments included: decreased spring snowpack via snow removal (Snow Removal: not heated, snow removal), warming via passive heating (Heated: heated, no removal), decreased spring snowpack and warming (Heated and Snow Removal: heated, snow removal), and control (Control: not heated, no removal). A best fit line was added to display the relationship between the variables, with bolded lines indicating a significant correlation.

Table 5: Multiple comparison of means with Tukey contrasts to identify whether treatment affected individual plant flowering onset, flowering duration, or maximum floral display. Each model included heated and snow removal treatments as main effects as well as their interaction with individual nested within plot as a random effect, and year as an additive random effect. Bolded p-values indicate significant effects at $\alpha < 0.05$.

Source	z-value	p-value
Flowering Onset		
heated	-2.94	**0.0171**
snow removal	-6.28	**<0.0001**
heated x snow removal	-5.61	**<0.0001**
Flowering Duration		
heated	3.43	**0.0035**
snow removal	3.73	**0.0011**
heated x snow removal	4.94	**<0.0001**
Maximum Floral Display		
heated	1.89	0.2302
snow removal	1.64	0.3570
heated x snow removal	2.78	**0.0273**

Table 6: Mixed effects models to identify whether treatment × year interactions affected individual plant flowering onset, flowering duration, or maximum floral display over time. The treatment × year interactions were tested to evaluate if treatment effects grew stronger over time. Each model included the interaction of year and heated and snow removal treatments with individual nested within plot as a random effect. Bolded p-values indicate significant effects at $\alpha < 0.05$.

Parameter	*Flowering Onset*		*Flowering Duration*		*Maximum Floral Display*	
	t-value	p-value	t-value	p-value	z-value	p-value
heated	4.991	**<0.0001**	-1.300	0.195	0.34	0.7338
snow removal	-0.714	0.4756	-0.095	0.925	-1.72	0.0852
heated x snow removal	-2.315	**0.0213**	1.151	0.250	-2.76	**0.0058**
year	-0.600	0.5492	-1.698	0.090	-3.76	**0.0002**
heated x year	-4.999	**<0.0001**	1.304	0.193	-0.33	0.739
snow removal x year	0.717	0.4737	0.093	0.926	1.72	0.0853
heated x snow removal x year	2.317	**0.0212**	-1.151	0.251	2.76	**0.0057**

Discussion

Climate change is affecting the phenology and flower production of flowering plants globally. Warmer temperatures and earlier snowmelt individually lead to advanced flowering onset (Arft et al. 1999, Fitter and Fitter 2002, Steltzer et al. 2009, Wipf 2010, Gezon et al. 2016, Wadgymar et al. 2018, Kehrberger and Holzschuh 2019, Jabis et al. 2020), extended flowering

durations (Price and Waser 1998, Dunne et al. 2003, Jabis et al. 2020), and greater maximum floral displays (Arft et al. 1999, Gezon et al. 2016), but the combined effects of warming and earlier snowmelt on flowering phenology are poorly understood, especially over many consecutive years. Consistent with our predictions, *B. sagittata* phenology and floral production varied in response to warming via passive heating *with* experimentally-accelerated spring snowmelt across the seven-year study, suggesting that components of climate change interact to affect montane floral populations and community interactions through altered phenology. Heating with snow removal drove the patterns of earlier flower onset, extended flowering duration, and increased maximum floral display – beyond the individual effects of heating and snow removal. These results suggest that earlier springs in montane regions will likely lead to earlier and longer flowering periods with more flowers produced, thereby reducing possible phenological mismatches with interacting species. Our prediction concerning the ability of heating with snow removal treatments to counteract each other with respect to flowering duration was not supported. The effects of heating with snow removal on earlier onset and increased maximum floral displays strengthened over time, indicating that species like *B. sagittata* may benefit from conditions of climate change and short-term experiments likely underestimate the potential impacts of warming temperatures and earlier snowmelt on flowering phenology.

Heating with Snow Removal Leads to Earlier Flowering Onset

Flowering onset occurred earlier under conditions of snow removal as well as heating with snow removal, suggesting that snow removal drives this trend. This result indicates that earlier access to light enables photosynthesis and fosters earlier growth and the onset of flowering of *B. sagittata,* rather than changes in temperature. While earlier flowering can result

from higher plant productivity due to increased soil moisture in snow removal plots in some systems (Lambert et al. 2010, Gezon et al. 2016), soil moisture in these snow removal plots was significantly reduced (Sherwood et al. 2017). Therefore, earlier light availability is more likely to drive the observed responses to snow removal than changes in soil moisture.

The effect of heating and heating with snow removal on earlier flower onset strengthened over the seven-year study period, signifying that the influence of temperature on flower onset became more important with time and may eventually outweigh the effects of snow removal long term. Together, these results indicate that while early spring snowmelt has a strong, consistent effect on *B. sagittata* flowering onset, the effects of warming on flowering onset have the potential to play a stronger role in directing flowering phenology over decades of climate change, especially as climate warming intensifies.

Heating with Snow Removal Extended Flowering Durations

Flowering duration was prolonged under conditions of snow removal and heating with snow removal, with longer durations related to earlier flower onset. Our results are consistent with previous work linking longer forb flowering durations to earlier snowmelt (Price and Waser 1998) and to increased soil heat (Dunne et al. 2003, Jabis et al. 2020). However, other studies have attributed shortened flower durations to warming (Kudo 2020) resulting from earlier senescence (Nagahama et al. 2018), likely due to increasing drought stress with declining soil moisture as the growing season progresses. This study system exhibited declining soil moisture following Day of Year (DOY) 170 (Sherwood et al. 2017), although from 2013 to 2019 *B. sagittata* individuals completed flowering by DOY 170 regardless of treatment. Notably, we found no change in the timing of flowering senescence across all treatments, similar to another

early-season forb, glacier lily (*Erythronium grandiflorum*) (Lambert et al. 2010). Research presented here combined the effects of heating with snow removal to uncover the interactive ability of these treatments to advance onset, maintain senescence date, and thereby extend flowering duration - allowing longer periods of flowering that facilitate interactions with other organisms in montane communities. It should be noted that *B. sagittata* would likely extend flowering durations in warmer years with earlier snowmelt due to its sequential flowering behavior. Research by Jabis et al. (2020) also found an interactive effect between snowmelt date and temperature, with earlier snowmelt and higher temperatures further lengthening flowering durations of forb species. Congruent with our results, durations are dependent on snowmelt date (Price and Waser 1998, Dunne et al. 2003, Kudo 2020). Additionally, our research showed that the effects of all treatments on flowering duration were consistent over time, suggesting that flowering durations remain stable in their responses to advanced spring snowmelt and warming.

Maximum Floral Display Increased Under Heating with Snow Removal

Maximum floral display (i.e., maximum number of concurrent flowers) marginally increased under conditions of heating with snow removal, indicating that the combination of these climatic variables influences montane plant investment in floral displays and potentially impacts their fitness. Our findings were consistent with previous research that observed increased flower numbers with earlier snowmelt (Gezon et al. 2016) and warming (Arft et al. 1999). By contrast, other studies have found decreased flower numbers with earlier snowmelt (Inouye 2008) and warming (Saavedra et al. 2003, Liu et al. 2012), suggesting that the effects of warming and advanced spring snowmelt on maximum floral display are context dependent on the species and location. Therefore, additional research is needed to evaluate the physiological

responses to warming and early snowmelt in relation to maximum floral displays of flowering species. Soil moisture affects maximum floral displays, with declining moisture leading to lower investments in reproduction, reflected in reduced flower production (Phillips et al. 2018). *Balsamorhiza sagittata* in our study system complete flowering prior to observed declines in soil moisture (Sherwood et al. 2017), therefore, soil moisture may be unlikely to affect *B. sagittata* floral production in a given growing season. Few other studies have investigated the potential for snow removal to interact with heating to affect maximum floral display. Our results indicate that heating with snow removal increases maximum floral display for *B. sagittata* and that this effect strengthens over time. These findings highlight the positive effect that warmer conditions and reduced snowpack could have on early-spring floral displays in montane meadows in the context of climate change.

Earlier Flower Onset Associated with Longer Flowering Durations and Greater Maximum Floral Displays

Our findings uncovered that flower onset was negatively correlated with flowering duration across all treatments, with the heated and snow removal treatment experiencing the strongest negative response. The consistency of this pattern across treatments, including the control, suggests that regardless of local resource availability (i.e., light, soil moisture), individuals of *B. sagittata* that flower early also flower for a long duration in this system. This pattern could also be indicative of overall cooler temperatures earlier in the spring allowing individual flower longevity to increase, therefore extending flowering durations (Yasaka et al. 1998). In addition, flowering duration was positively correlated with maximum floral display, with an especially strong relationship in the snow removal treatment. This strong correlation suggests that *B. sagittata* plants allocate resources towards increasing flower production when

flowering for longer periods in response to earlier light access. This pattern between flowering duration and floral display has been found more generally in plants as a way to evade herbivory and increase fitness (Ehrlén et al. 2015). For the heated and heated with snow removal treatments, the lack of correlation suggests that there may not be a trade-off between flowering longer and producing more flowers based on resources available. For instance, healthy and/or larger plants may bloom longer and produce more flowers, while unhealthy and/or smaller plants may bloom for shorter periods of time and produce fewer flowers. Additionally, due to *B. sagittata*'s sequential flowering behavior, extended flowering durations may occur among individuals with greater floral displays. Finally, flowering onset was negatively correlated with maximum floral display across treatments, with the control eliciting the strongest negative response. This pattern implies that the timing of flowering onset may dictate the size of *B. sagittata* maximum floral display, with individuals that flower earlier allocating resources prior to competitors and enabling the production of more flowers to attract pollinators and maximize fitness. Earlier flowering leading to increased seed production (i.e., increased fitness) has been supported by previous research on the early spring flowering species, *Mertensia fusiformis* (Forrest and Thomson 2010). This relationship of earlier flowering and resource allocation to produce more flowers may indicate *B. sagittata's* resiliency to short-term summer dry periods and a lack of pollinator interactions due to asynchrony (Forrest and Thomson 2010). Further, it may indicate that climate change may have weaker negative effects on *B. sagittata* plant fitness, the pollinators with which *B. sagittata* interacts, and overall *B. sagittata* community dynamics than expected.

Potential Implications to Plant-Pollinator Interactions

Advancement in flowering onset in response to earlier spring snowmelt and long-term warming may result in a phenological mismatch where floral resources emerge prior to pollinators (Walther et al. 2002, Parmesan and Yohe 2003, Kudo and Ida 2013). Such mismatches could have cascading effects on plant reproduction, impacting populations of both plants (Kudo and Ida 2013) and their pollinators, especially specialists (Memmott et al. 2007), in the long term (Kehrberger and Holzschuh 2019). Patterns of earlier flower onset and extended flowering duration could mitigate phenological mismatches with interacting pollinators because these conditions provide a wider window of time for possible interaction with floral resources. However, it would be essential to determine the degree of a temporal shift in emergence and duration for both the plants and associated pollinators to effectively establish the severity of a potential mismatch (Rafferty et al. 2015). Increased maximum floral display in response to heating and/or snow removal may have more complex impacts on interacting pollinators that warrant additional investigation. For example, in the same system as our study under heating conditions, *B. sagittata* produced more flowers but these flowers had decreased nectar volume and increased sugar concentration (McCombs et al. manuscript submitted for publication). A review of the physiological effects of warming on plant-pollinator interactions (Scaven and Rafferty 2013) highlights that altered nectar production and composition can have immediate effects on pollinator visitation as reflected in pollinator energetics (Kudo and Harder 2005). Long-term consequences for pollinator fitness have been observed with greater nectar-sugar concentration associated with increased larval solitary bee growth (Burkle and Irwin 2009) and some butterflies and wasps relying on nectar for nutrients like amino acids and sugars (Kevan and Baker 1983). The patterns we observed in our experiment imply that real changes in

temperature and timing of snowmelt could have long-lasting impacts on plant-pollinator interactions, community structure and function, and ecosystem dynamics.

Conclusions

This research identified that the flowering phenology of *B. sagittata* responded most strongly to the combination of heating with snow removal rather than snow removal or heating only. Responses included advancing flower onset, extending flowering duration, and increasing maximum floral display. As one of the first studies to monitor the *combined* effects of snow removal and heating on montane flowering plants over a seven-year period, we observed that advanced spring snowmelt drives spring flowering plant phenology in montane ecosystems. Further, our results show that long-term studies are needed to quantify the effects of climate change because the influences of heating with snow removal that advanced flower onset and increased maximum floral display also strengthened over time.

This study provides important insights in understanding how climatic trends may affect the phenology and floral production of a long-lived, spring-flowering perennial plant species in montane regions. Our results indicate that the effects of climate change on floral phenology in this system could result in earlier flowering, extended flowering periods, and more flower production, which would contribute to our understanding of plant-pollinator interactions in montane ecosystems following snowmelt. This and similar montane systems are particularly susceptible to environmental cues and shifts in the timing of those cues (Cleland et al. 2007). Future research may consider using similar methodologies to determine if the patterns observed in this study hold for other long-lived, spring-flowering as well as mid-summer flowering montane perennials. Future conservation efforts to preserve native plants and their essential

interactions with pollinators will benefit from knowledge of how these species respond to changes in climate.

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CHAPTER FOUR

ROCKY MOUNTAIN FORBS EXHIBIT SPECIES SPECIFIC PHENOLOGICAL AND PHOTOSYNTHETIC RESPONSES TO HEATING

Abstract

Floral phenology, morphology, and photosynthetic performance are greatly affected by global climate change. Changes in the timing of environmental cues that initiate species morphology, physiology, and behavior patterns may lead to phenological shifts in a species' typical life cycle pattern affecting biological events. Rising temperatures have direct effects on plant physiology, where increasing temperatures increase photosynthesis and respiration rates to an optimum and then decline with increasing temperatures beyond a species' optimum leading to decreased photosynthetic performance and plant survival. Extensive work has been conducted evaluating the effects of climate change on native flowering plant phenology and morphology as well as the photosynthetic performance (i.e., chlorophyll fluorescence) of crops, cultivars, and trees. This research sought to evaluate gaps in existing knowledge about phenology, morphology, photosynthetic performance, the relationships between them, and local abiotic environmental conditions effects on photosynthetic performance in three native flowering species in the Rocky Mountain Region of the USA. The effects of passive heating on the flower onset, flowering duration, maximum floral display, and chlorophyll fluorescence (F_V/F_M) of *Achillea millefolium*, *Geranium viscosissimum*, and *Penstemon procerus* individuals were tested over the course of two consecutive growing seasons. Overall, species-specific responses of flowering duration, maximum floral display, and photosynthetic performance were observed in response to the

heating treatment. Flowering durations of *Achillea* and *Geranium* were extended, and *Geranium* produced more flowers in the heating treatment. There was no effect of heating on F_V/F_M, but species varied in F_V/F_M with *Achillea* displaying the greatest photosynthetic performance followed by *Geranium* and *Penstemon*. These findings suggest that *Achillea* and *Geranium* are well adapted to heat stress. Decreasing mean F_V/F_M was correlated with longer *Achillea* flowering durations and larger *Geranium* maximum floral displays, indicating a potential tradeoff between photosynthetic performance and reproduction. Maximum soil moisture, maximum soil temperature, maximum air temperature, and maximum surface temperature were the best local abiotic environmental condition predictors of F_V/F_M. *Achillea* and *Geranium* displayed tolerance to warming conditions with longer flowering duration, more flowers produced, and higher photosynthetic performance. Therefore, we encourage the use of *Achillea millefolium* and *Geranium viscosissimum* in plant rehabilitation efforts, erosion control, and pollinator restoration strategies in the Rocky Mountain Region.

Introduction

Climate change is a growing global pressure on floral phenology (Walther et al. 2002) triggering local to ecosystem level responses. Changes in the timing of environmental cues that initiate species morphology, physiology, and behavior patterns may lead to phenological shifts in a species' typical life cycle pattern affecting biological events (Parmesan and Yohe 2003, Cleland et al. 2007). For instance, plant phenology dictates the timing of the growing season, thus determining annual carbon uptake in terrestrial ecosystems (Gu et al. 2003). Further, variation in phenological life-cycle events across species, thereby reducing resource competition, in diverse communities is vital to maintain species coexistence (Rathcke and Lacey 1985). A meta-analysis

found that a warming climate has caused advancements in plant germination, leaf emergence, flowering and fruiting, and green up (Parmesan and Hanley 2015). Five long-term phenological datasets across the Northern Hemisphere and spanning hundreds of years detected earlier spring phenology (i.e., budding, flowering, leaf-out) in response to increasing spring temperatures, with rapid acceleration of this effect since 1985 (Vitasse et al. 2022). Additionally, changing precipitation regimes may also alter plant phenology. A meta-analysis on the effects of extreme and seasonal precipitation on plant growth and flowering found that in mesic environments, plant growth decreased in response to extreme precipitation, and reduced spring and summer precipitation altered phenology with reduced fruit production and leaf development (Zeppel et al. 2014). Monitoring plant phenology over time is an effective tool in evaluating climatic changes and shifts in ecosystem productivity (Cleland et al. 2007).

Another way to evaluate global climate change effects is investigating plant photosynthetic performance in response to a changing environment. Temperature has direct effects on plant physiology, where increasing temperatures increase photosynthesis and respiration rates to an optimum and then begins to decline with increasing temperatures beyond a species' optimum leading to decreasing plant survival (Halofsky et al. 2018). Research assessing the thermotolerance of *Pinus ponderosa* and *Pseudotsuga menziesii* needles found that at about 40°C photosynthesis efficiency rapidly declines and at about 55°C goes to zero (Marias et al. 2017a), indicating extreme heat limits photosynthetic performance. A growth chamber experiment assessing heat stress durations effects on *Coffea arabica* recovery found that photosynthesis significantly declined two days after a 49°C warming treatment (Marias et al. 2017b). The recovery of photosynthesis took longer for plants exposed to the warming treatment

for a longer period of time (45 minutes vs 90 minutes) (Marias et al. 2017b). Further, increasing heat stress led to reduced water-use efficiency regardless of heat stress duration (Marias et al. 2017b). Exposure to higher temperatures, even if over a shorter duration, appears to often induce a stronger response than does long-term moderate warming (Jagadish et al. 2021).

Water is one of three resources needed by plants to conduct photosynthesis, therefore, water availability is important for photosynthetic performance. Generally, as late summer approaches water availability begins to dwindle due to evaporation of water from the soil to the atmosphere and transpiration of water from plants to the atmosphere (i.e., evapotranspiration). This leaves plants at risk of water stress depending on their morphological adaptations. Some plants have evolved large taproots to access water year-round and other plants have evolved large root networks to acquire any water that may be in the vicinity. However, not all plants evolved in environments where it was necessary to adapt to declining water availability and they are now facing the effects of anthropogenic climate change reflected in newly declining water supplies. These plants face water scarcity and dehydration leading to declining photosynthesis and eventual mortality. The relationship between temperature and water usage on plant photosynthetic performance have direct implications at the individual plant and population level with indirect effects on the ecosystem and can be assessed using chlorophyll fluorescence.

Chlorophyll fluorescence assesses plant damage and predicts subsequent plant performance by evaluating the light dependent reaction of photosynthesis, which is highly sensitive to stress (Ritchie 2006). This is done by analyzing the fluorescence emissions emanating from chlorophyll A in photosystem II of the light dependent reaction (Ritchie 2006). Many of the excited electrons from chlorophyll A in photosystem II are not captured by the

mobile electron carrier protein and they decay back to their ground state (Ritchie 2006). It is the energy lost in this decay process that gives off fluorescent light which is measured in chlorophyll fluorescence (Ritchie 2006). The measurements are taken by, first, acclimating plant cells to darkness for several minutes to deplete the excited electrons in the leaf/plant (Ritchie 2006). Then the plants and plant cells are exposed to a brief pulse of high intensity photosynthetically active light to monitor the rise and fall of fluorescence emissions with a sensitive photometer (Ritchie 2006). That process produces a relative fluorescence curve over time made up of four values: F_O, F_M, F_V, and F_T. F_O represents the initial fluorescence emissions where all reactions centers are open, and F_M is the maximum fluorescence point. F_V is the variable fluorescence or the rise from F_O to F_M and F_T is the steady state after the gradual decay of emissions following F_M (Ritchie 2006). The ratio of F_V/F_M is the direct measurement of a plant's optimal quantum efficiency, which indicates the efficiency of the light dependent reaction (Ritchie 2006). The theoretical maximum value of F_V/F_M is 0.83, indicating healthy photosynthetic performance of a plant (Björkman and Demmig 1987, Ritchie 2006). F_V/F_M values below 0.83 could be an indication of stress with values below 0.6 clearly indicating plant stress (Ritchie 2006). Plants that are damaged or stressed can recover quickly, which is why it is important to measure chlorophyll fluorescence parameters, like F_V/F_M, over several days following stress events to draw proper inferences about plant damage and/or stress (Ritchie 2006).

Plant physiology likely influences some metrics of plant phenology and morphology throughout the growing season. However, few studies have evaluated the link between photosynthetic performance and phenological metrics, like flower onset, flowering duration, and flower production. Research evaluating heat stress on *Coffea arabica* using F_V/F_M found that for

both of the heating treatments (45-minutes at 49°C and 90-minutes 49°C), there was a reduction in F_V/F_M and flower production was prevented (Marias et al. 2017b), suggesting that heat stress even in short durations can lead to crop or plant population failure. With regards to a warming climate, heat stress causes shifts in the allocation of assimilated carbon to repair and recovery processes rather than growth and reproduction. Therefore, during times of heat stress plants will likely reduce the number of flowers produced and reduce their nectar supply as these are carbon costly productions for the plant. A field experiment conducted in Sicily investigating the effects of shading on chlorophyll fluorescence of two species of *Trifolium* found increasing F_V/F_M values until flowering onset (Mauro et al. 2011), suggesting a reproduction tradeoff following optimal photosynthetic performance. There is a clear gap in existing knowledge and conflicting results associated with investigating the relationship between photosynthetic performance and flower onset, flowering duration, and flower production, with most research conducted on crop species.

Native flowering plant phenology and flower production responses to a changing climate have been studied extensively, but little research has been conducted evaluating photosynthetic responses of native species. Earlier flower onset in response to warmer temperatures (Arft et al. 1999, Fitter and Fitter 2002, Kehrberger and Holzschuh 2019, Jabis et al. 2020) and advanced snowmelt (Steltzer et al. 2009, Wipf 2010, Gezon et al. 2016, Wadgymar et al. 2018, Jabis et al. 2020) has been documented across native species. Warming temperatures have led to shortened flowering durations resulting from earlier senescence (Nagahama et al. 2018, Kudo 2020). Conversely, extended flowering durations have been observed in response to warming and early snowmelt (Price and Waser 1998, Dunne et al. 2003, Jabis et al. 2020). Various levels of flower

production have been documented with warming resulting in increases (Arft et al. 1999) and decreases (Saavedra et al. 2003, Liu et al. 2012) and advanced snowmelt leading to increases (Gezon et al. 2016) and decreases due to spring frost events (Inouye 2008). The combination of warming and advanced spring snowmelt has led to earlier flower onset, extended flowering durations, and more flowers produced (Durney et al. 2022). Increases in winter temperatures initiated earlier growth, erratic bud break, and increased the risk of spring frost fatality (Tixier et al. 2019). The photosynthetic performance of crops, cultivars, and trees in response to a changing environment has been studied intensively (Baker and Rosenqvist 2004, Guanter et al. 2014, Duarte et al. 2016, Marias et al. 2017a, b, Wan et al. 2020), but little research has been done evaluating native flowering plants.

This research sought to evaluate gaps in existing knowledge about photosynthetic performance, phenology, morphology and the relationships between them in native flowering species in the Rocky Mountain Regions of the USA. Therefore, three flowering species were selected for this research based on the following criteria, (1) native and abundant in the western United States, (2) vary in morphology from one another, (3) important for pollinator communities in the western United States, and (4) cultivated species and commercially available. Based on those four criteria, *Achillea millefolium*, *Geranium viscosissimum*, and *Penstemon procerus* were the three native forb species selected. *Achillea millefolium* (common yarrow) is the most widely distributed native perennial flower species in the western United States residing in a variety of habitats and flowering from May to early fall (Mann et al. 2022). *Achillea* are in the Asteraceae family and have dome shaped to flat topped flowering heads forming clusters of white disk flowers. *Achillea* ranges from sea level to 3350 m and are capable of surviving in

numerous climates, plant communities, and soil types (Mann et al. 2022). This species is used in conservation practices in the United States for restoration efforts and erosion control (Mann et al. 2022). Additionally, *Achillea* attracts numerous wildlife species including beneficial insects, small mammals, and ungulates (Mann et al. 2022). *Geranium viscosissimum* (sticky geranium) is a native forb perennial widely distributed in the western United States ranging from 300 m to 3200 m elevations among foothills, canyons, open woodlands, and montane environments (Scheinost and Stannard 2010). *Geranium* are among the Geraniaceae family producing dispersed clusters of saucer-shaped pink to magenta flowers blooming from May through August (Scheinost and Stannard 2010, Delphia et al. 2016). This species is frequented by flies, butterflies, and numerous species of bees (Scheinost and Stannard 2010, Delphia et al. 2016). *Penstemon procerus* (littleflower penstemon) is a native perennial forb found in the western United States ranging from middle to high elevations among gravelly ridges, open rocky slopes, grassy hillsides, dry meadows, open woods, and alpine zones (Bauer 1983, Krampien and Low 2020). These *Penstemon* are among the Plantaginaceae family and have flowers with blue-purple fused-tubular shaped petals arranged in a raceme and bloom June through August (Krampien and Low 2020, Maron and Marler 2007). This species has been known to attract pollinators of various tongue lengths (Bauer 1983), including bees, butterflies, hummingbirds, and the *Penstemon* specialist, *Osmia brevis* (Krampien and Low 2020).

The guiding questions of this research were (1) how does heating affect the flower onset, flowering duration, maximum floral display, and photosynthetic performance of three prominent flowering plants in the Rocky Mountains? (2) how do local abiotic environmental conditions affect and help predict photosynthetic performance (i.e., F_V/F_M)? and (3) what is the relationship

between mean F_V/F_M and flower onset, flowering duration, and maximum floral display?

Hypotheses for each of these questions can be found in Table 7.

Table 7: Hypotheses about how *Achillea millefolium*, *Geranium viscosissimum,* and *Penstemon procerus* flower onset, flowering duration, maximum floral display (i.e., maximum number of concurrent flowers), and mean chlorophyll fluorescence may respond to passive heating. Additionally, hypotheses about how *Achillea millefolium*, *Geranium viscosissimum,* and *Penstemon procerus* chlorophyll fluorescence responds to passive heating and various local temperature and moisture environmental conditions. Explanatory variables are listed in the left column and response variables are listed on the top row.

Response ➡ Explanatory ⬇	FLOWER ONSET	FLOWERING DURATION	MAXIMUM FLORAL DISPLAY	F_V/F_M
HEAT	Earlier in response to increased soil heat	Extended in response to increased soil heat	Increase in response to earlier flowering onset and extended flowering duration	Decrease in response to heat stress
HEAT ACROSS SPECIES	Earlier in response to increased soil heat	Extended in response to increased soil heat	Increase in response to earlier flowering onset and extended flowering duration	Similar decline in response to heat stress
HEAT BETWEEN YEARS	The effect of heat will strengthen over the 2-year study	The effect of heat will strengthen over the 2-year study	The effect of heat will strengthen over the 2-year study	The effect of heat will strengthen over the 2-year study
ENVIRONMENTAL CONDITIONS	Not evaluated in this paper	Not evaluated in this paper	Not evaluated in this paper	Increasing temperatures will lower F_V/F_M values due to increasing heat stress
HEAT * MEAN F_V/F_M	Earlier in response to increased soil heat	Shortened in response to heat stress	Decrease in response to heat stress	Not evaluated

Materials & Methods

Study Site

This study was conducted in Bozeman, Montana, USA at Montana State University's Horticulture Farm (45.663709°; -111.072004°) at an elevation of 4906 m during the 2020 and 2021 growing seasons. The study site area was tilled a month prior to the study onset. Mean monthly temperatures in Bozeman, MT vary from 10 °C in May to 19 °C in July (Western Regional Climate Center). Mean monthly precipitation during the growing season varies from 3.2 cm in August to 7.4 cm in June (Western Regional Climate Center).

Experimental Methods and Design

Twelve 2.4 m^2 (8 ft X 8 ft) plots were established in 2020 and monitored May to September in 2020 and 2021. Plots were spaced 4.5 m apart on the north and south sides and 6 m apart on the east and west sides (Figure 9). Heat was manipulated to create two treatments (N = 6 replicates/treatment): warming via passive heating (H: heating) and control (C: not heated). Treatment assignments randomly alternated by plot (Figure 9). Treatments assigned to plots remained the same for the duration of the study. Heating was accomplished by constructing a 2.4-m^2 (8 ft X 8 ft) passive heating structure. The top of the structure was 59 cm from the ground. The passive heating structures were made of polyvinyl chloride pipe with a glass-house plastic roof with small perforations allowing precipitation to fall through to the ground. All passive heating structures enhanced the downward reflectance of infrared radiation on the ground and increased the daily minimum temperatures of plant and soil surfaces by 2 °C (Sherwood et al. 2017). Mean surface temperatures in western North America are projected to increase by 2–5 °C by 2100 (Halofsky et al. 2018, IPCC 2021). The passive heating structures used here increase

surface temperature on the low end of regional climate projections; therefore, resulting responses to warming are a conservative response. Passive heating structures were installed on designated plots in early May prior to the growing season and remained in place until early to mid-September after the growing season was completed. Soil temperatures and moisture for each plot were recorded every hour throughout the growing season using a soil temperature/moisture probe buried 25 cm below the surface. Surface temperatures for each plot were recorded every hour throughout the growing season using HOBO loggers placed on the soil surface in the center of each plot. The ambient air temperature was recorded for the set of plots every hour throughout the growing season using an air sensor placed 50 cm above the soil surface outside of the plots.

One hundred and twenty individuals of each of the three species were purchased as seedlings from Westscape Wholesale Nursery in Belgrade, MT, USA in May 2020. Within each of the twelve plots, ten individuals of each species were planted alternating species by column and row (Figure 10). Transplanting of all individual plants and treatment application took place mid-May 2020. Plants were supplementally watered every three days throughout the first growing season (i.e., 2020) to ensure that plants would successfully transplant. Supplemental watering did not take place during the second growing season (i.e., 2021). Plots were weeded by hand every two weeks throughout the growing season to reduce competitive effects with other plant species invading the study plots.

Individual plants were numbered with metal identification tags and monitored during the growing season during the two years of this study. All individual plants within each of the 12 plots were measured every 3 days from emergence (mid-May) to senescence (end of August). Measurements included whether the individual was dead or alive and, if alive, the number of

flowers and their phenological stage (i.e., buds, fully flowering, senescing, and senesced). Individual flowers were counted instead of counting the number of inflorescences, since the number of individual flowers per inflorescence varied within a species and between species. Flowering heads were considered fully open once petals were open enough for a pollinator to access the stamens, senescing once petals withered, and senesced once seed maturity was observed. We distinguished among these stages because fully open and senescing flowers are the only stages that provide nectar resources for pollinators (Potts et al. 2003), and we wanted to quantify this window of time for each treatment. Flowering onset (i.e., date of first flower) was defined as the date on which an individual plant was observed to have at least one fully opened flower. Duration of flowering was defined as the number of days between observation of an individual's first fully open flower and its last senescing flower in a given growing season. Maximum floral display (i.e., the maximum number of concurrent flowers) was calculated by totaling the number of fully flowering, senescing, and senesced flowers of an individual at each visit to identify the maximum number of flowers present concurrently on an individual in a given year. Buds were not included in these counts because the number of flowers produced was intended as a proxy for the quantity of available nectar, and buds that failed to flower provided no nectar. The sum of all flowers produced by an individual over the growing season would not be reliable due to the potential for the same flower to be counted multiple times on successive visits. Using the maximum number of concurrent flowers eliminates the possibility of recounting the same flowers, providing a conservative estimate for the number of flowers produced by an individual over each growing season. Additionally, disintegrated and fallen senesced flowers were excluded when using the maximum number of concurrent flower metrics.

Chlorophyll fluorescence (F_V/F_M) was measured every 3 days in 2020 and every 6 days in 2021. One leaf per plant individual was measured while dark-adapted (i.e., predawn) using a MINI-PAM-II Photosynthesis Yield Analyzer from Walz Germany. In the event the sun rose prior to completing the F_V/F_M measurements - sampling was terminated for that sampling day. Chlorophyll fluorescence was measured as the ratio of variable to maximum fluorescence (F_V/F_M) to assess the maximum quantum efficiency of photosystem two (PSII) (Maxwell and Johnson 2000), which is calculated as:

$$\frac{F_V}{F_M} = \frac{F_M - F_O}{F_M}$$

Statistical Methods Evaluating Flower Onset, Flowering Duration, and Maximum Floral Display Responses to Heating

To examine the effect of passive heating on flower onset, flowering duration, and maximum floral display, we used mixed-effects models with treatment as a fixed effect. Individual plant identity nested within plot was included as a random effect to account for multiple measurements of each individual across plots over the 2-year duration of this study. Additionally, year was included as a crossed additive random effect to control for random year-to-year variation around the patterns of the treatments. This allowed us to assess how treatment affected each variable while accounting for year-to-year differences in weather and other conditions. All statistical analyses for flower onset, flowering duration, and maximum floral display were conducted for each species as each species has a unique life history. For maximum floral display, generalized linear mixed-effects models with a negative binomial distribution to account for overdispersion were fit. Analyses of variance were used to test significant differences in the means between treatment groups (i.e., passive heating and control). All analyses were

evaluated at a significance level of 0.05, where $p < 0.05$ indicates significance, $0.05 < p < 0.1$ indicates marginal significance, and $p > 0.1$ indicates no significance.

To test whether treatment effects on flower onset, flowering duration, and maximum floral display took time to develop (cumulative effects of treatment) or grew stronger over time, year was included as an interaction with treatment as fixed effects in each mixed-effects model. Initial evidence of an interaction between treatment and year was supported by an F test prior to further analysis and interpretation. Pairwise comparisons of treatment–year combinations are not shown because absolute values of treatment effects depended on year.

Statistical Methods Evaluating F_V/F_M Response to Heating

For all analyses of F_V/F_M, measured F_V/F_M values of 1.0 are considered to have no damage with decreasing values indicating increasing heat stress. Statistical analyses were performed to determine whether F_V/F_M of individual plants differed between species, differed between the heated treatment and the control across the 2-year study, and whether the effect of treatment grew stronger over time. Additionally, statistical analyses were performed to determine whether F_V/F_M of individual plants differed by year and differed by species for each year.

To examine the effect of passive heating on F_V/F_M, we used mixed-effects models with treatment, date, and species as fixed effects. Individual plant identity nested within plot was included as a random effect to account for multiple measurements of each individual across plots over the 2-year duration of this study. Analyses of variance were used to test significant differences in means between treatment and species groups. All analyses were evaluated at a significance level of 0.05, where $p < 0.05$ indicates significance, $0.05 < p < 0.1$ indicates marginal significance, and $p > 0.1$ indicates no significance.

To test whether treatment effects on F_V/F_M took time to develop (cumulative effects of treatment) or grew stronger over time, date was included as an interaction with treatment along with species as fixed effects in each mixed-effects model. Individual plant identity nested within plot was included as a random effect to account for multiple measurements of each individual across plots over the 2-year duration of this study.

To test whether treatment effects on F_V/F_M differed by year, year was included as an interaction with treatment along with species as fixed effects in each mixed-effects model. Individual plant identity nested within plot was included as a random effect to account for multiple measurements of each individual across plots over the 2-year duration of this study.

To determine whether treatment effects on F_V/F_M for each species differed by year, year was included as an interaction with treatment in a mixed-effects model using data subset by species. Individual plant identity nested within plot was included as a random effect to account for multiple measurements of each individual across plots over the 2-year duration of this study.

Statistical Methods Evaluating Which Environmental Conditions are the Best Predictors of F_V/F_M

Temperature and moisture variables were collected on site using HOBO loggers and soil dataloggers to identify influential environmental condition variables affecting F_V/F_M. The list of environmental variables included: minimum, maximum, mean, median soil temperature (°C), minimum, maximum, mean, median soil moisture ($m^3\ m^{-3}$), minimum, maximum, mean, median air temperature (°C), minimum, maximum, mean, median soil surface temperature (°C) from 2020 and 2021 growing seasons (Table 8). These environmental variables were selected based on their availability of instrument collection.

Correlation tests were conducted between all explanatory environmental condition variables to prevent the inclusion of highly correlated variables in models, which could lead to multicollinearity and unreliable estimates. Uncorrelated moisture and temperature explanatory variables were included in each generalized linear model. Then, generalized linear models using the *glm* function in R were fit to evaluate which explanatory environmental condition variables were the most influential in explaining F_V/F_M. AIC comparison between models was conducted to determine the most influential explanatory variables. Following generalized linear models, interactions between the most influential temperature and moisture explanatory variables were used to evaluate potential interactive effects between environmental condition variables on F_V/F_M. Final AIC comparison, including a null model, was conducted to determine the best model to describe F_V/F_M.

Statistical Methods Evaluating Flower Onset, Flowering Duration, and Maximum Floral Display Responses to Mean F_V/F_M

Mixed-effects models were used to test the interactive effects of treatment and mean F_V/F_M on flower onset, flowering duration, and maximum floral display and included individual plant identity nested within plot as random effects to account for multiple measurements of each individual across plots over the 2-year duration of this study. Year was included as a crossed additive random effect in each mixed-effects model to control for random year-to-year variation around the patterns of the treatments. This allowed us to assess how the interaction of treatment and mean F_V/F_M affected each variable while accounting for year-to-year differences in weather and other conditions. All statistical analyses for flower onset, flowering duration, and maximum floral display were conducted by species as each species have unique life histories from one

another. Analyses of variance were used to test significant differences in the means between treatment groups.

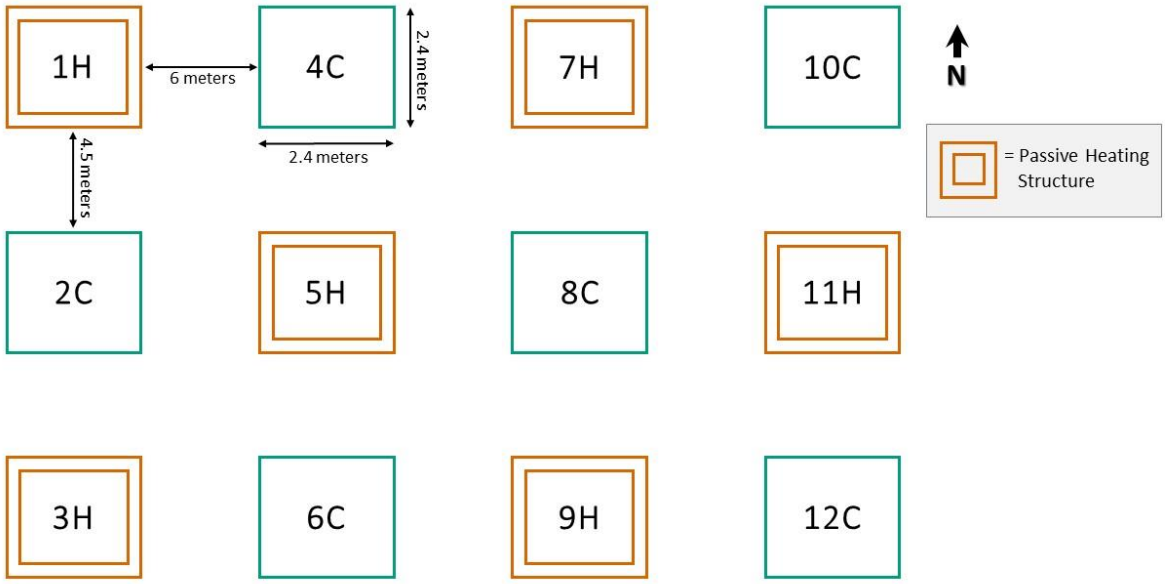


Figure 9: Plot layout where H represents the heating treatment using passive heating structures (N = 6) and C represents the control treatment (N = 6). Plots were 2.4 m^2 (8 ft X 8 ft), 59 cm tall, and spaced 4.5 m apart on the north and south sides and 6 m apart on the east and west sides. Green represents control plots and brown represents heating plots.

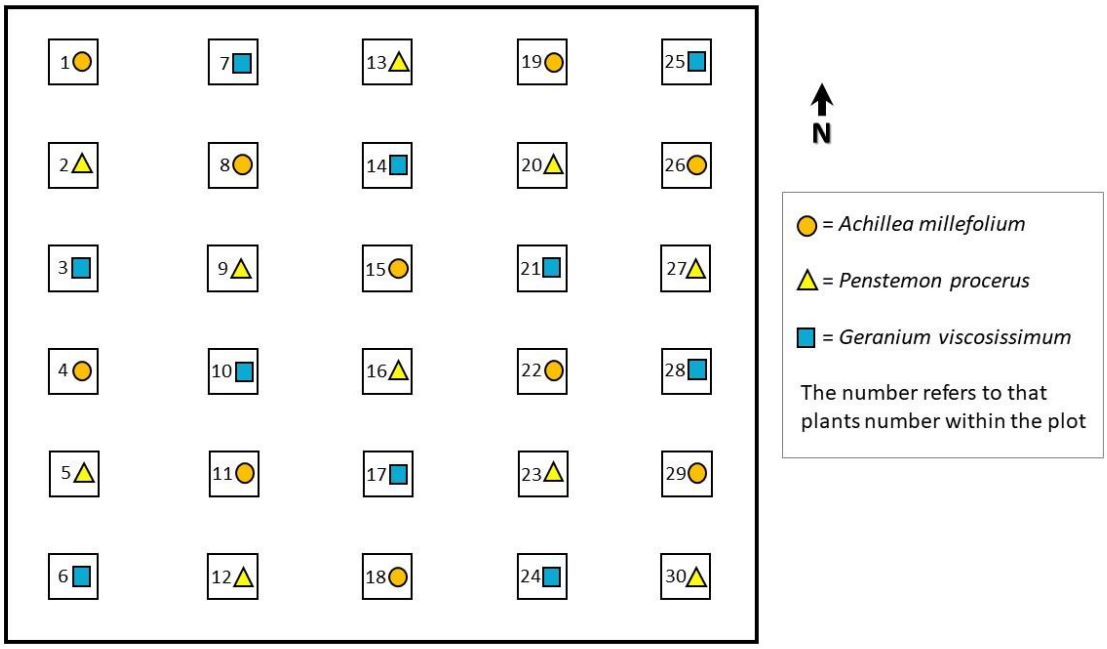


Figure 10: Plant layout within each plot which included 30 individuals total with 10 individuals for each of the three species. The three species included *Achillea millefolium* (orange circle), *Geranium viscosissimum* (blue square), and *Penstemon procerus* (yellow triangle). Each number corresponds to that plants individual identification number within each plot.

Table 8: Temperature and moisture environmental condition variables collected on site using HOBO loggers and soil dataloggers to identify predictor environmental condition variables affecting F_V/F_M. Listed metrics derived from each temperature and moisture environmental condition variable.

List of local environmental variables collected	Metrics derived
Soil Temperature (°C)	Minimum, Maximum, Mean, Median
Soil Moisture (m^3/m^3)	Minimum, Maximum, Mean, Median
Air Temperature (°C)	Minimum, Maximum, Mean, Median
Soil Surface Temperature (°C)	Minimum, Maximum, Mean, Median

Results

Neither flower onset nor maximum floral display differed between the heated treatment and the control group for all species (Figure 11, Table 9). The heated treatment significantly affected flowering duration of *Achillea* and *Geranium* but not *Penstemon*, with flowering

durations extending five to six days longer in the heated treatment compared to the control (Figure 11, Table 9). Flower onset differed significantly by year for all three species, with earlier onset occurring in 2021 compared to 2020 (Figure 12, Table 10). *Achillea* flowering duration differed significantly with the interactive effect of treatment and year with the longest flowering duration occurring in the heated treatment in 2021 at 69 days and the shortest flowering duration occurring in the heated treatment in 2020 at 35 days (Figure 12, Table 10). The flowering duration of *Geranium* also differed by treatment and year with significantly longer durations occurring in the heated treatment and in 2021 at 44 days, but the interactive effect of treatment and year was insignificant (Figure 12, Table 10). *Penstemon* flowering duration differed significantly by year with longer flowering durations occurring in 2021 at about 36 days regardless of treatment (Figure 12, Table 10). Maximum floral display differed significantly by year for *Achillea* and *Penstemon* with increased floral displays in 2021 regardless of treatment (Figure 12, Table 10). In contrast, the interactive effect of treatment and year lead to significantly greater maximum floral displays of *Geranium* in the heated treatment in 2020 and the greatest maximum floral displays in 2021 regardless of treatment (Figure 12, Table 10).

F_V/F_M differed significantly by species and date (Table 11), with the highest F_V/F_M for *Achillea* followed by *Geranium* and *Penstemon* regardless of date (Figure 13). There was no effect of treatment on F_V/F_M (Figure 13, Table 11). A significant interactive effect of treatment and year for each species on F_V/F_M was detected (Figure 14, Table 12). *Achillea* and *Penstemon* did not differ between treatments but differed by year with greater F_V/F_M values in 2020 compared to 2021 (Figure 14, Table 12). In contrast, *Geranium* F_V/F_M values differed by year

with greater values in 2020 than in 2021, additionally, the control group in 2021 had significantly greater F_V/F_M values than the heated treatment (Figure 14, Table 12).

There was a significant effect of species, date, and the interactive effect of treatment and date on F_V/F_M, with no significant treatment effect (Table 13). F_V/F_M decreased over time with the highest F_V/F_M values in the heated treatment in 2020 decreasing into 2021 with the heated treatment F_V/F_M values being lower than the control F_V/F_M values in 2021 (Figure 15). This trend was the same when species data was subset and analyzed separately (Figure 16, Table 14).

Based on constructed generalized linear models for each variable and AIC comparisons among models for each variable (i.e., minimum soil temperature vs. maximum soil temperature vs. mean soil temperature vs. median soil temperature), the best environmental condition predictors of F_V/F_M were maximum soil temperature, maximum soil moisture, maximum air temperature, and maximum surface temperature (Table 15). Then, model selection was conducted by constructing 8 linear mixed effects models with combinations of maximum soil temperature, maximum soil moisture, maximum air temperature, and maximum surface temperature, their interactions, and treatment to determine the best model (Table 16). Each model was compared to a null model (i.e., $F_V/F_M \sim 1$). AIC comparison between models identified that maximum soil temperature, maximum soil moisture, the interactive effect of maximum soil temperature and maximum air temperature, and the interactive effect of maximum soil temperature and maximum soil surface temperature were the best predictors of F_V/F_M with increasing F_V/F_M in response to increasing soil temperature and soil moisture (Figures 17 & 18, Tables 16 & 17). Mean maximum soil moisture, mean maximum soil temperature, mean maximum air temperature, and mean maximum surface temperature by mean F_V/F_M across

species indicated that *Achillea* maintained a higher mean F_V/F_M followed by *Geranium* and then *Penstemon* across all abiotic variables (Figure 19).

Mean F_V/F_M, treatment, and the interaction between mean F_V/F_M and treatment had no significant effects on flower onset for all species (Table 18). While mean F_V/F_M alone did not significantly affect Achillea flowering duration, treatment and the interactive effect of mean F_V/F_M and treatment had a significant effect on *Achillea* flowering duration (Table 18) with decreasing mean F_V/F_M coinciding with increased flowering duration (Figure 20). Treatment had a significant effect on *Geranium* flowering duration as determined in previous analysis above (Figure 11, Tables 9 & 18). *Penstemon* flowering durations were not significantly affected by mean F_V/F_M, treatment, or the interactive effect of mean F_V/F_M and treatment (Table 18). Mean F_V/F_M and the interactive effect of mean F_V/F_M and treatment significantly affected *Geranium* maximum floral displays (Table 18) with increased maximum floral displays coinciding with lower F_V/F_M values (Figure 20). *Achillea* and *Penstemon* maximum floral displays were not significantly affected by mean F_V/F_M, treatment, or the interactive effect of mean F_V/F_M and treatment (Table 18).

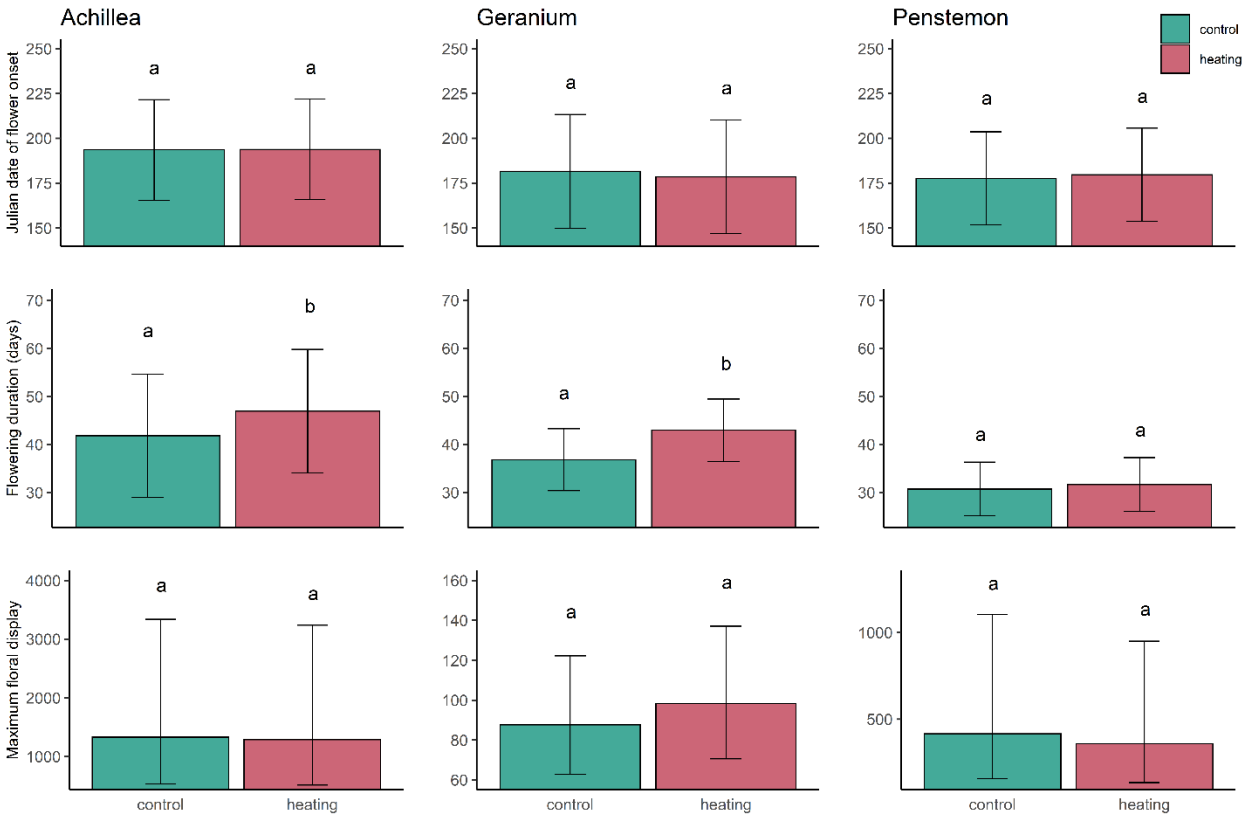


Figure 11: Effect of treatment on the least squares mean flower onset date, flowering duration, and maximum floral display for *Achillea*, *Geranium*, and *Penstemon* accounting for both years. Treatments included heating via passive heating and control. Estimated marginal mean pairwise comparison is indicated by letters where the same letter indicates overlap and different letters indicate variation. The error bars represent the upper and lower 95% confidence levels.

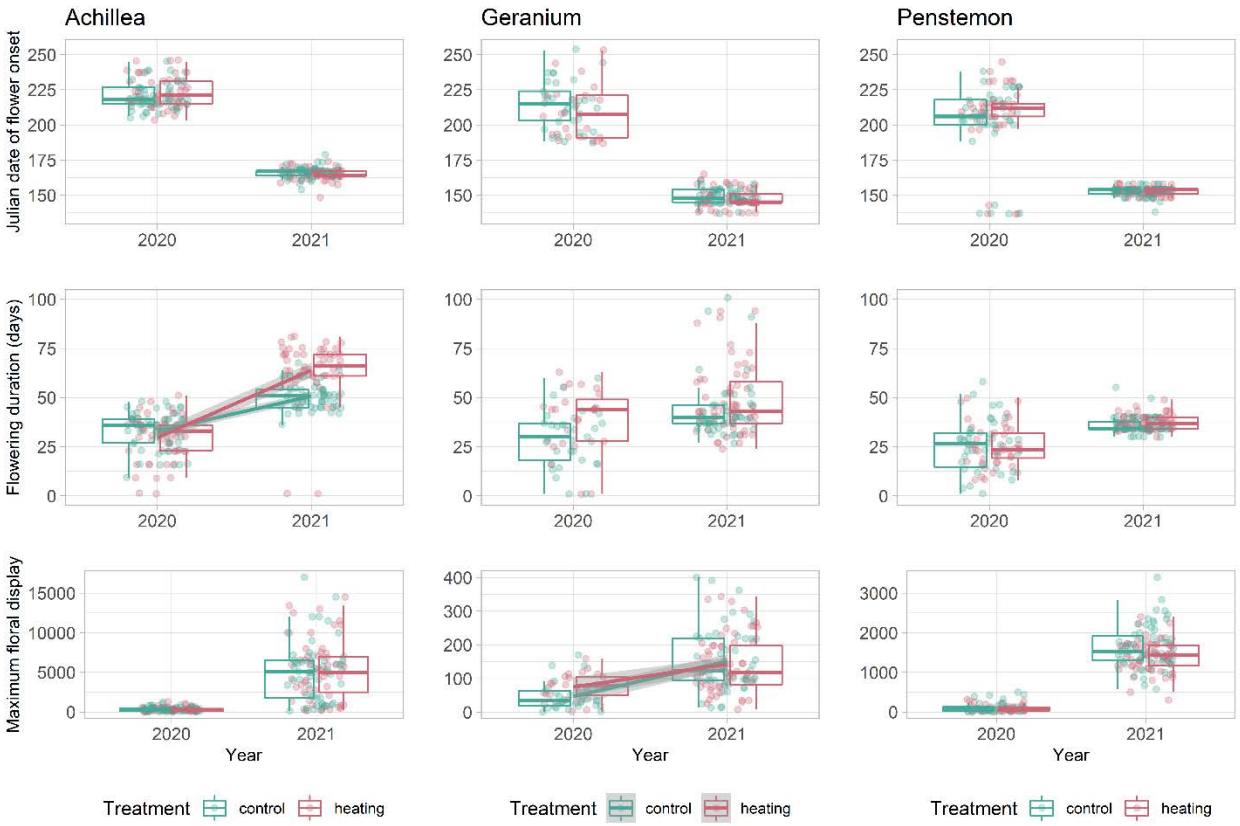


Figure 12: Treatment effects between 2020 and 2021 on flower onset, flowering duration, and maximum floral display for *Achillea*, *Geranium*, and *Penstemon*. Treatments included heating via passive heating and control. The figure displays results of treatment interactions with year. Lines indicate a significant treatment × year interaction (indicated by treatment color) compared with the control on the response variable (i.e., flowering onset, flowering duration, and maximum floral display). The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear change in each treatment over time. There was a significant difference in flower onset, flowering duration, and maximum floral display for each species between 2020 and 2021.

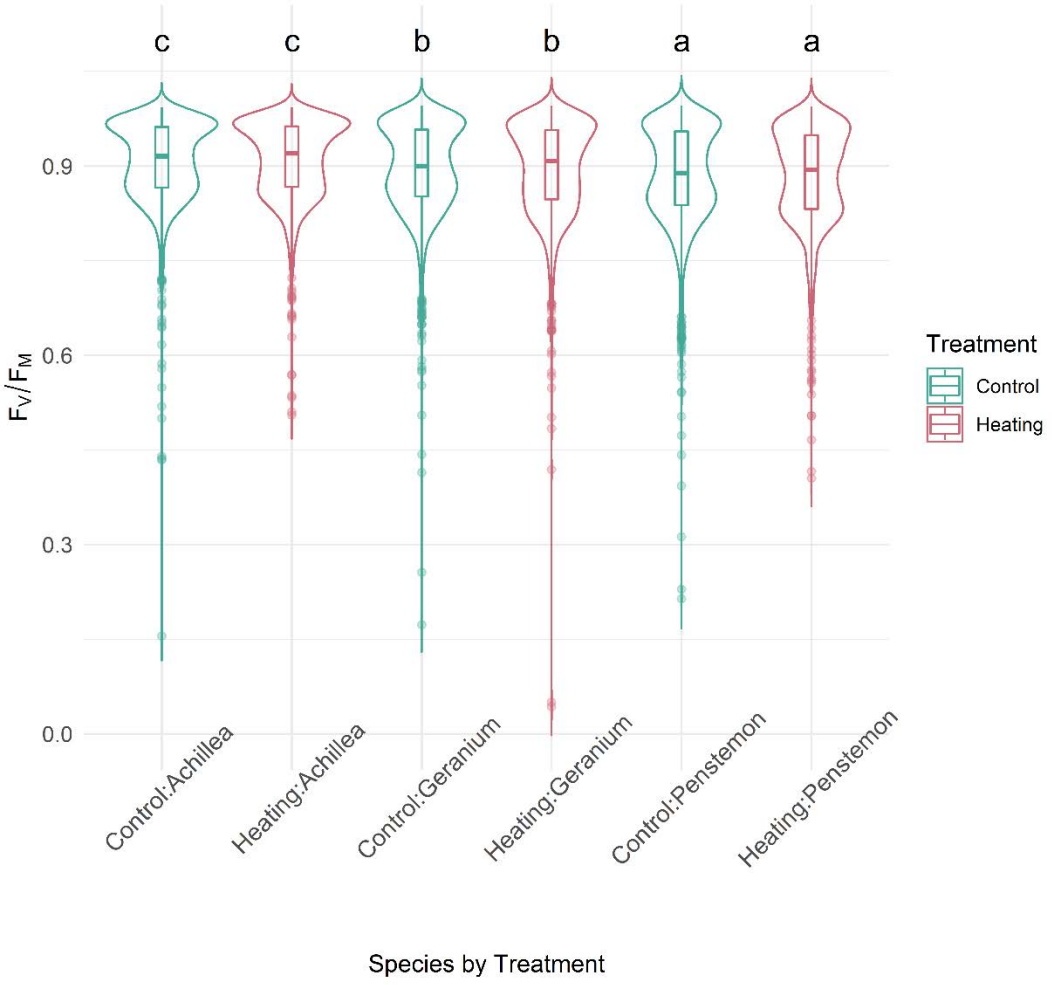


Figure 13: Effect of treatment on the least squares mean F_V/F_M for *Achillea*, *Geranium*, and *Penstemon*. Treatments included heating via passive heating and control. Estimated marginal mean pairwise comparison is indicated by letters where the same letter indicates overlap and different letters indicate variation. The boxplot error bars represent the upper and lower 95% confidence levels.

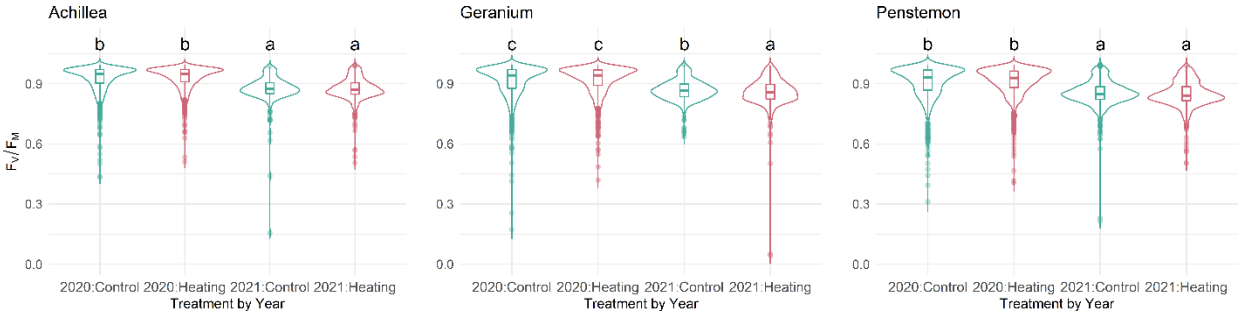


Figure 14: Effect of treatment between 2020 and 2021 on the least squares mean F_V/F_M for *Achillea*, *Geranium*, and *Penstemon*. Treatments included heating via passive heating and control. Estimated marginal mean pairwise comparison is indicated by letters where the same letter indicates overlap and different letters indicate variation. The boxplot error bars represent the upper and lower 95% confidence levels.

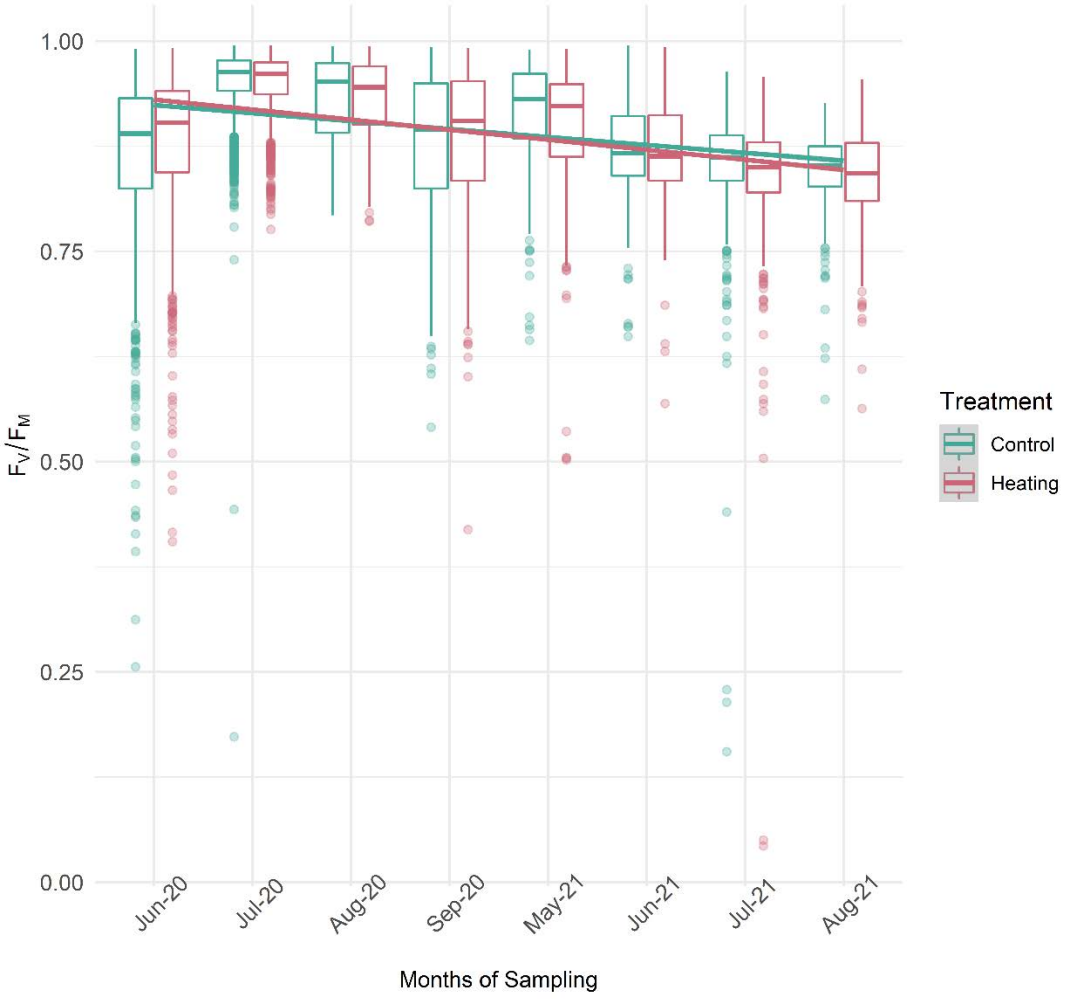


Figure 15: Treatment effects during the growing seasons for the two-year study on F_V/F_M of *Achillea*, *Geranium*, and *Penstemon*. Treatments included heating via passive heating and control. The figure displays results of treatment interactions with sampling date. Lines indicate a significant treatment × date interaction (indicated by treatment color) compared with the control on F_V/F_M. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear change in each treatment over time. There was a significant difference in F_V/F_M for each species over time.

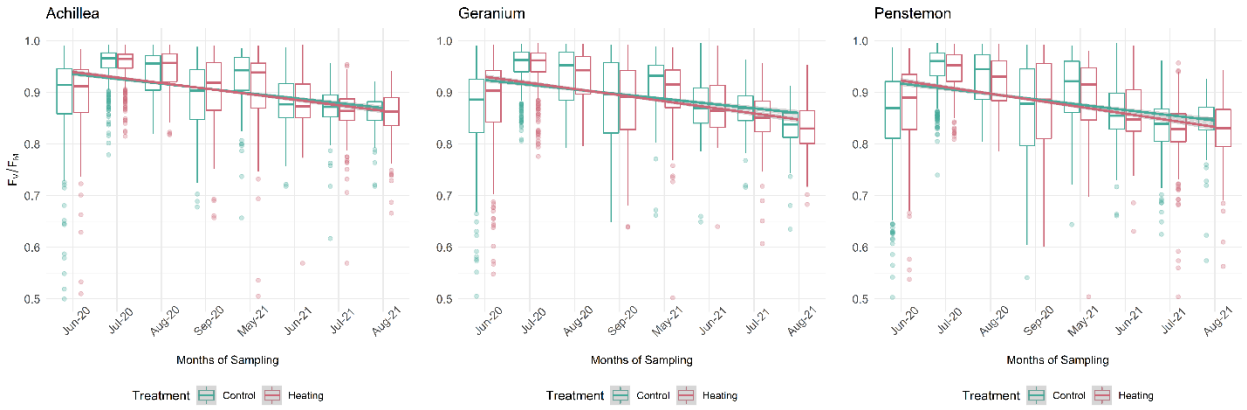


Figure 16: Treatment effects during the growing seasons for the two-year study on F_V/F_M for each species (*Achillea*, *Geranium*, and *Penstemon*). Treatments included heating via passive heating and control. The figure displays results of treatment interactions with sampling date. Lines indicate a significant treatment × date interaction (indicated by treatment color) compared with the control on F_V/F_M. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear change in each treatment over time. There was no significant difference in F_V/F_M between treatments.

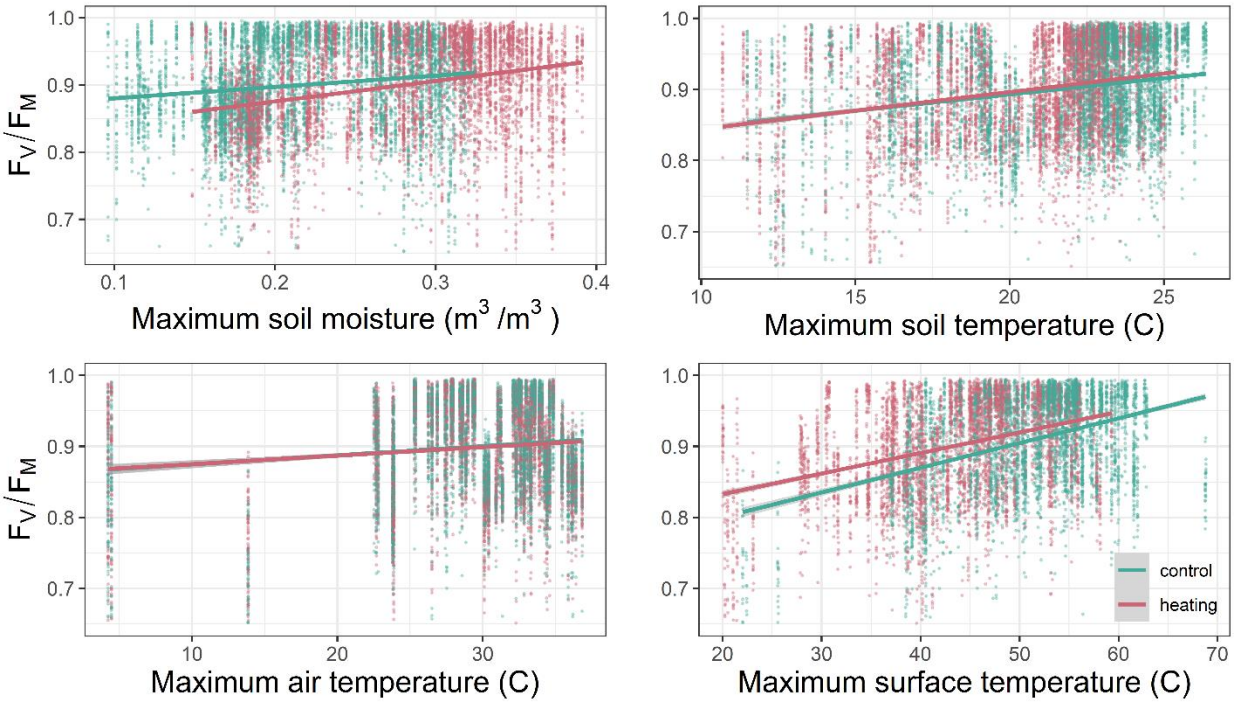


Figure 17: Relationships between best predictive temperature and moisture variables (maximum soil moisture, maximum soil temperature, maximum air temperature, maximum surface temperature) from best model and F_V/F_M across all species (*Achillea*, *Geranium*, and *Penstemon*). Treatments included heating via passive heating and control. Lines indicate a significant effect of that variable on F_V/F_M. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear relationship. Lines color coded by treatment to evaluate differences between the two treatments. There was no significant difference in F_V/F_M between treatments.

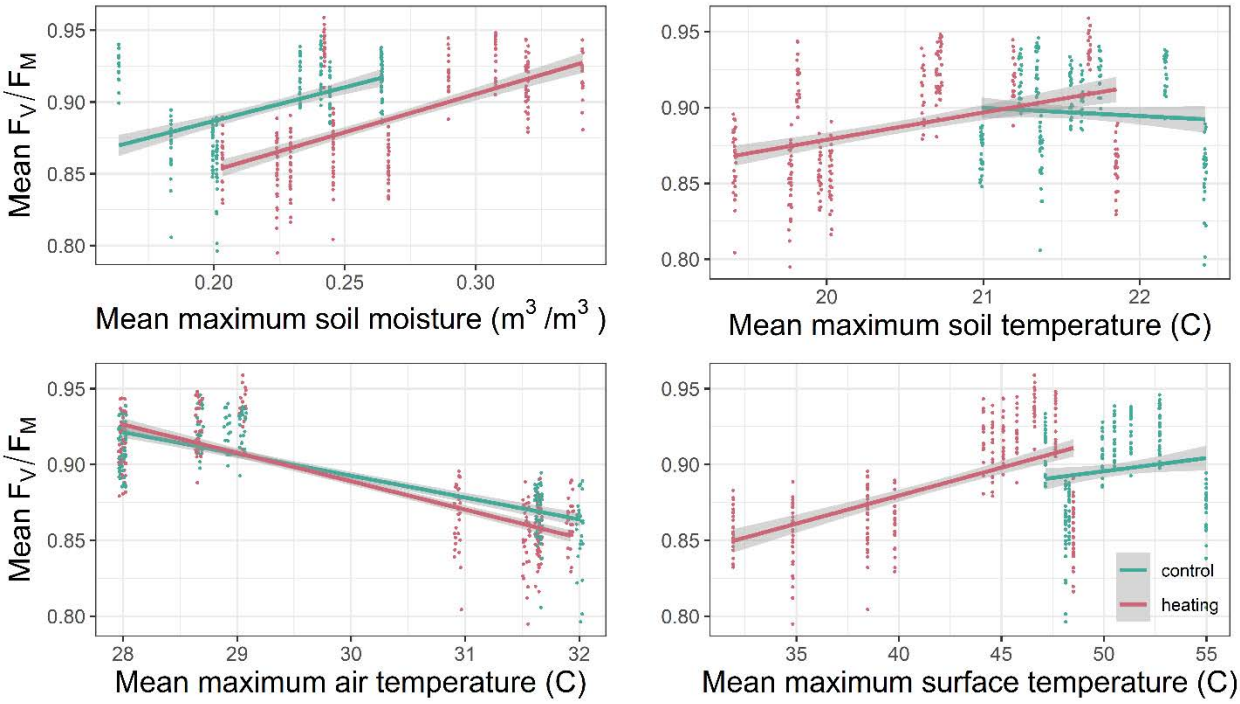


Figure 18: Relationships between mean maximum soil moisture, mean maximum soil temperature, mean maximum air temperature, mean maximum surface temperature and mean F_V/F_M across all species (*Achillea*, *Geranium*, and *Penstemon*). Treatments included heating via passive heating and control. Lines were added to display the relationship between the variables and mean F_V/F_M. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear relationship. Lines color coded by treatment to evaluate differences between the two treatments. There was no significant difference in mean F_V/F_M between treatments when each of the following abiotic variables were included in analysis of variance tests: mean maximum soil moisture test – Treatment: $F_{1,13} = 0.31$, p value = 0.5870; mean maximum soil temperature test – Treatment: $F_{1,13} = 0.20$, p value = 0.6590; mean maximum air temperature test – Treatment: $F_{1,9} = 0.07$, p value = 0.7991; mean maximum surface temperature test – Treatment: $F_{1,16} = 2.24$, p value = 0.1539.

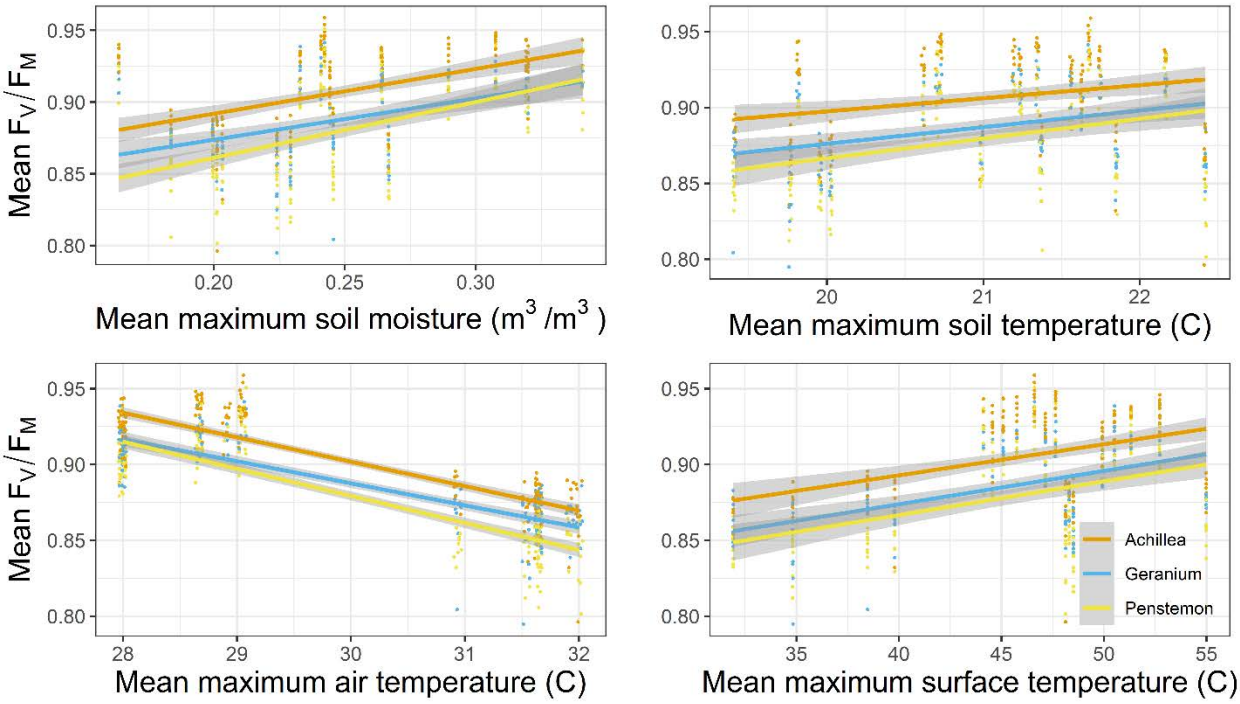


Figure 19: Relationships between mean maximum soil moisture, mean maximum soil temperature, mean maximum air temperature, mean maximum surface temperature and mean F_V/F_M across species (*Achillea*, *Geranium*, and *Penstemon*). Lines were added to display the relationship between the variables and mean F_V/F_M. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear relationship. Lines color coded by plant genus to evaluate differences between the three plant species. There were significant differences in mean F_V/F_M across plant species when each of the following abiotic variables were included in analysis of variance tests: mean maximum soil moisture test – Genus: $F_{2,274} = 101.49$, p value = <0.0001; mean maximum soil temperature test – Genus: $F_{2,275} = 99.12$, p value = <0.0001; mean maximum air temperature test – Genus: $F_{2,275} = 102.25$, p value = <0.0001; mean maximum surface temperature test – Genus: $F_{2,275} = 98.44$, p value = <0.0001.

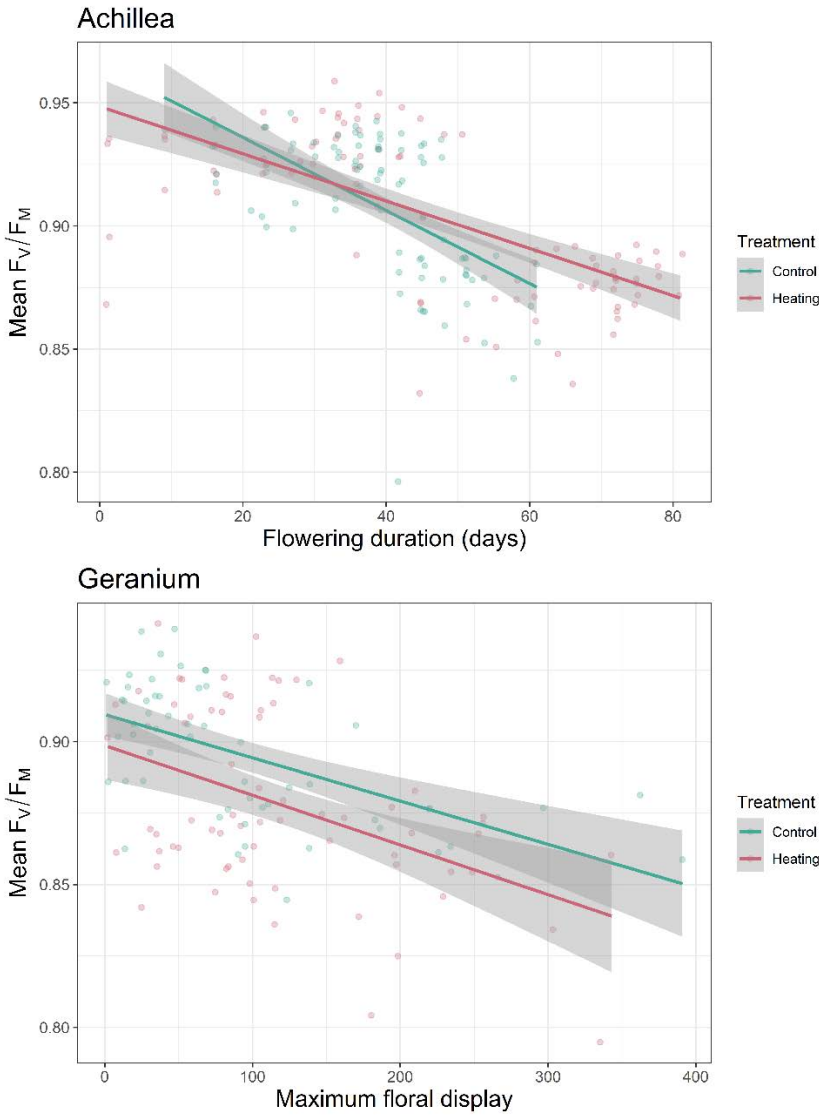


Figure 20: Significant relationships between mean F_V/F_M, *Achillea* flowering duration, and *Geranium* maximum floral display. Lines indicate a significant mean F_V/F_M × treatment interaction (indicated by treatment color) compared with the control on flowering duration and maximum floral display. The normal confidence intervals (indicated by the gray-shaded areas along each line) were constructed using generalized linear smoothing to observe a linear relationship. Lines color coded by treatment to evaluate differences between the two treatments. There was no significant difference in mean F_V/F_M between treatments.

Table 9: Analysis of variance and deviance results testing the effect of treatment (heating versus control) on Julian date of flower onset, flowering duration in days, and maximum floral display (i.e., maximum number of concurrent flowers) for *Achillea*, *Geranium*, and *Penstemon*. Bolded p values indicate significant effects at $\alpha < 0.05$.

Response	**Species**	**F**	**DF**	**P**
FLOWER ONSET	Achillea	0.08	1,10	0.7804
	Geranium	2.48	1,10	0.1471
	Penstemon	0.72	1,10	0.4157
FLOWERING DURATION	Achillea	9.15	1,10	**0.0130**
	Geranium	5.77	1,10	**0.0377**
	Penstemon	0.48	1,10	0.0503
		Chisq	**DF**	**P**
MAXIMUM FLORAL DISPLAY	Achillea	0.04	1	0.8486
	Geranium	1.14	1	0.2853
	Penstemon	1.31	1	0.2532

Table 10: Analysis of variance results testing the effect of treatment (heating versus control), year, and the interactive effect of treatment and year on Julian date of flower onset, flowering duration in days, and maximum floral display (i.e., maximum number of concurrent flowers) for *Achillea*, *Geranium*, and *Penstemon*. Bolded p values indicate significant effects at $\alpha < 0.05$.

Response	**Source**	**F**	**DF**	**P**
FLOWER ONSET				
Achillea	Treatment	0.08	1,10	0.7799
	Year	2915.16	1,111	**<0.0001**
	Treatment*Year	2.26	1,111	0.1359
Geranium	Treatment	2.55	1,10	0.1419
	Year	1090.65	1,96	**<0.0001**
	Treatment*Year	0.79	1,97	0.3760
Penstemon	Treatment	0.71	1,10	0.4200
	Year	468.79	1,109	**<0.0001**
	Treatment*Year	0.67	1,109	0.4162
FLOWERING DURATION				
Achillea	Treatment	9.73	1,10	**0.0110**
	Year	282.76	1,111	**<0.0001**
	Treatment*Year	29.58	1,111	**<0.0001**
Geranium	Treatment	5.74	1,10	**0.0383**

	Year	23.37	1,94	<**0.0001**
	Treatment*Year	0.35	1,95	0.5545
Penstemon	Treatment	0.49	1,10	0.4994
	Year	90.96	1,109	<**0.0001**
	Treatment*Year	0.17	1,109	0.6778
MAXIMUM FLORAL DISPLAY		**Chisq**	**DF**	**P**
Achillea	Treatment	0.32	1	0.5692
	Year	264.26	1	<**0.0001**
	Treatment*Year	0.45	1	0.5026
Geranium	Treatment	6.10	1	**0.0135**
	Year	59.98	1	<**0.0001**
	Treatment*Year	5.58	1	**0.0181**
Penstemon	Treatment	0.83	1	0.3629
	Year	361.80	1	<**0.0001**
	Treatment*Year	0.003	1	0.9574

Table 11: Analysis of variance testing the effect of treatment (heating versus control), species (*Achillea*, *Geranium*, and *Penstemon*), and sampling date on F_V/F_M. Bolded p values indicate significant effects at $\alpha < 0.05$.

Response	**F**	**DF**	**P**
Treatment	0.02	1,10	0.8929
Species	167.25	2,342	<**0.0001**
Date	380.66	36,12113	<**0.0001**

Table 12: Analysis of variance testing the effect of treatment (heating versus control), year (2020 versus 2021), and the interactive effect of treatment and year on F_V/F_M for each species (*Achillea*, *Geranium*, and *Penstemon*). Bolded p values indicate significant effects at α <0.05.

Species	**Source**	**F**	**DF**	**P**
Achillea	Treatment	0.15	1,10	0.7106
	Year	779.43	1,4092	**<0.0001**
	Treatment*Year	4.74	1,4092	**0.0295**
Geranium	Treatment	0.16	1,10	0.6996
	Year	556.37	1,4035	**<0.0001**
	Treatment*Year	10.85	1,4035	**0.0010**
Penstemon	Treatment	0.02	1,10	0.8882
	Year	669.91	1,4132	**<0.0001**
	Treatment*Year	4.14	1,4132	**0.0420**

Table 13: Analysis of variance testing the effect of treatment (heating versus control), species (*Achillea*, *Geranium*, and *Penstemon*), sampling date, and the interactive effect of treatment and sampling date on F_V/F_M for each species. Bolded p values indicate significant effects at α <0.05.

Response	**F**	**DF**	**P**
Treatment	0.02	1,10	0.8909
Species	166.80	2,342	**<0.0001**
Date	395.33	36,12077	**<0.0001**
Treatment*Date	13.98	36,12077	**<0.0001**

Table 14: Analysis of variance testing the effect of treatment (heating versus control), sampling date, and the interactive effect of treatment and sampling date on F_V/F_M for each species (*Achillea*, *Geranium*, and *Penstemon*). Bolded p values indicate significant effects at α <0.05.

Species	**Source**	**F**	**DF**	**P**
Achillea	Treatment	0.52	1,10	0.4867
	Date	133.40	36,3928	**<0.0001**
	Treatment*Date	5.30	36,3928	**<0.0001**
Geranium	Treatment	0.07	1,10	0.8015
	Date	129.06	36,3944	**<0.0001**
	Treatment*Date	5.90	36,3944	**<0.0001**
Penstemon	Treatment	0.03	1,118	0.8523
	Date	151.63	36,4069	**<0.0001**
	Treatment*Date	7.09	36,4069	**<0.0001**

Table 15: AIC comparison of generalized linear models fit to evaluate which correlated explanatory environmental condition variable metrics were the most informative in explaining F_V/F_M. Models compared include metrics for each environmental condition variable (i.e., soil temperature, soil moisture, air temperature, and surface temperature). Bold values indicate the lowest AIC and its associated environmental condition variable metric.

	SOIL TEMPERATURE		SOIL MOISTURE		AIR TEMPERATURE		SURFACE TEMPERATURE	
	DF	AIC	DF	AIC	DF	AIC	DF	AIC
MINIMUM	3	85178.57	3	85085.72	3	85376.52	3	68441.18
MAXIMUM	3	**84455.11**	3	**85084.03**	3	**85091.50**	3	**66799.45**
MEAN	3	84916.15	3	85085.68	3	85377.39	3	67180.01
MEDIAN	3	84970.90	3	85086.81	3	85349.30	3	67821.43

Table 16: AIC comparison of generalized linear models fit with interactions between the most informative temperature and moisture explanatory variables used to evaluate potential interactive effects between environmental condition variables on F_V/F_M. Bold values indicate the best model with the lowest AIC.

Model	DF	AIC
F_V/F_M ~ Maximum Soil Temperature + Maximum Soil Moisture + Maximum Soil Temperature*Maximum Air Temperature + Maximum Soil Temperature*Maximum Surface Temperature	**10**	**65427.01**
F_V/F_M ~ Maximum Soil Temperature*Maximum Soil Moisture + Maximum Soil Temperature*Maximum Air Temperature + Maximum Soil Temperature*Maximum Surface Temperature	11	65428.24
F_V/F_M ~ Maximum Soil Temperature*Maximum Soil Moisture + Maximum Soil Temperature + Maximum Air Temperature + Maximum Soil Temperature*Maximum Surface Temperature	10	65455.15
F_V/F_M ~ Maximum Soil Temperature*Maximum Soil Moisture + Maximum Soil Temperature*Maximum Air Temperature + Maximum Soil Temperature + Maximum Surface Temperature	10	65560.54
F_V/F_M ~ Maximum Surface Temperature	5	66543.11
F_V/F_M ~ Maximum Soil Temperature	5	84247.55
F_V/F_M ~ Maximum Soil Moisture	5	84861.90
F_V/F_M ~ Maximum Air Temperature	5	84896.15
F_V/F_M ~ 1	2	85424.05

Table 17: Analysis of variance results testing the effect of maximum soil temperature, maximum soil moisture, maximum air temperature, maximum surface temperature, the interactive effect of maximum soil temperature and maximum air temperature, the interactive effect of maximum soil temperature and maximum surface temperature, and treatment on F_V/F_M. Bolded p values indicate significant effects at $\alpha < 0.05$.

Variables in Best Model	**F**	**DF**	**P**
Maximum Soil Temperature	221.20	1,9453	**<0.0001**
Maximum Soil Moisture	395.64	1,5265	**<0.0001**
Maximum Air Temperature	81.37	1,9951	**<0.0001**
Maximum Surface Temperature	589.38	1,7870	**<0.0001**
Treatment	1.45	1,633	0.2282
Maximum Soil Temperature*Maximum Air Temperature	39.64	1,9935	**<0.0001**
Maximum Soil Temperature*Maximum Surface Temperature	199.37	1,9935	**<0.0001**

Table 18: Analysis of variance results testing the effect of Mean F_V/F_M, treatment (heating versus control), and the interactive effect of Mean F_V/F_M and treatment on Julian date of flower onset, flowering duration in days, and maximum floral display (i.e., maximum number of concurrent flowers) for *Achillea*, *Geranium*, and *Penstemon*. Bolded p values indicate significant effects at $\alpha < 0.05$.

Response	**Source**	**F**	**DF**	**P**
FLOWER ONSET				
Achillea	Mean F_V/F_M	1.70	1,164	0.1934
	Treatment	0.12	1,9	0.7390
	Mean F_V/F_M *Treatment	0.99	1,102	0.3224
Geranium	Mean F_V/F_M	0.29	1,122	0.5878
	Treatment	2.68	1,9	0.1316
	Mean F_V/F_M *Treatment	0.46	1,82	0.4987
Penstemon	Mean F_V/F_M	1.65	1,132	0.2003
	Treatment	0.76	1,8	0.4045
	Mean F_V/F_M *Treatment	0.52	1,92	0.4734
FLOWERING DURATION				
Achillea	Mean F_V/F_M	0.02	1,165	0.8827
	Treatment	8.04	1,9	**0.0181**
	Mean F_V/F_M *Treatment	20.73	1,105	**<0.0001**
Geranium	Mean F_V/F_M	0.08	1,32	0.7756
	Treatment	5.91	1,9	**0.0344***
	Mean F_V/F_M *Treatment	0.39	1,82	0.5317
Penstemon	Mean F_V/F_M	0.02	1,103	0.8769
	Treatment	0.53	1,8	0.4859
	Mean F_V/F_M *Treatment	1.42	1,91	0.2354

MAXIMUM FLORAL DISPLAY		**Chisq**	**DF**	**P**
Achillea	Mean F_V/F_M	0.50	1	0.4799
	Treatment	0.06	1	0.8053
	Mean F_V/F_M *Treatment	0.0004	1	0.9831
Geranium	Mean F_V/F_M	54.51	1	**<0.0001**
	Treatment	0.13	1	0.7171
	Mean F_V/F_M *Treatment	10.71	1	**0.0011**
Penstemon	Mean F_V/F_M	3.82	1	0.0507
	Treatment	1.28	1	0.2577
	Mean F_V/F_M *Treatment	0.19	1	0.6670

Discussion

Overall, our findings determined that flower onset, flowering duration, maximum floral display, and photosynthetic performance (i.e., F_V/F_M) responses between the two years of the study were species specific with the heating treatment having no consistent effect among species (Figures 11 - 16). This differs from our hypotheses, assuming each species would respond to the heated treatment with earlier onset, extended flowering durations, increases in maximum floral displays and decreased F_V/F_M. Flowering duration was extended for *Achillea* and *Geranium* in heated plots (Figure 11) suggesting that these two species are better adapted to heat stress than *Penstemon* and even thrive under heated conditions. F_V/F_M differed by species with *Achillea* having the highest F_V/F_M value followed by *Geranium* and *Penstemon* (Figure 13), further indicating the adaptation heat stress of *Achillea* and *Geranium* (Scheinost and Stannard 2010, Mann et al. 2022). Flower onset and maximum floral displays were unaffected across species in response to heating (Figure 11) suggesting that our heating treatment may not have been strong enough to invoke a response. Flower onset, flowering duration, and maximum floral displays differed between years with earlier flowering occurring in 2021 regardless of treatment or

species, with *Achillea* and *Geranium* driving these patterns (Figure 12). Flowering duration was extended in 2021 with *Achillea* individuals experiencing longer flowering durations in the heated treatment due to the interactive effect of treatment and year, while *Geranium* and *Penstemon* did not vary by the interactive effect of treatment and year (Figure 12). Maximum floral displays increased across species in 2021, with *Geranium* producing slightly more (i.e., ~1) flowers generally across the heated treatment plots (Figure 12). These findings between year one and two are likely a biased result due to plants being transplanted in May 2020 and transplanting was not accounted for in models.

Previous research supporting our findings has clearly observed that optimum photosynthetic performance in response to temperature is species specific (Halofsky et al. 2018). F_V/F_M across species decreased between the two years with 2021 having the lowest F_V/F_M (Figure 14). This finding suggests that photosynthetic performance may decrease over the course of a plant's life or more likely that the lack of supplemental watering in 2021 led to increased water stress and decreased photosynthetic performance. *Geranium* F_V/F_M differed by treatment in 2021 with lower F_V/F_M in the heated treatment (Figure 14), indicating that *Geranium* may have been the only species to respond to the heated treatment with decreased photosynthetic performance – refuting heat stress resilience observations of the species relative to the other species assessed (Scheinost and Stannard 2010). F_V/F_M differed by the interactive effect of date and treatment with decreasing F_V/F_M consistent between treatments over the collective growing seasons for the two-year study (Figure 15). This finding could suggest photosynthetic performance regardless of treatment decreases over the course of a plant's life or that the lack of supplemental watering in 2021 led to increased water stress and decreased photosynthetic

performance. It should be noted that F_V/F_M values were lowest in June 2020 and July 2020 compared the rest of the growing seasons (Figure 15). As the plants were transplanted in May 2020, a low F_V/F_M value in June 2020 is understandable since plants were likely adjusting to a new environment. The low F_V/F_M values in June 2021 seem appropriate considering the slightly elevated F_V/F_M values in May 2021 and display a downward trend in photosynthetic performance throughout the 2021 growing season. Consistently across species, F_V/F_M interacted with year leading to lower F_V/F_M values in 2021 than in 2020 (Figure 16). In 2020, mean surface temperature was 22.9 °C, the temperature range of surface temperature was 68.9 °C, and mean soil moisture was 0.23 m^3/m^3. In 2021, mean surface temperature was 20.4 °C, the temperature range of surface temperature was 78.8 °C, and mean soil moisture was 0.21 m^3/m^3 (Table 19). It is likely that temperature fluctuations were driving decreasing photosynthetic performance trends (i.e., lower F_V/F_M values) in 2021 as the surface temperature range was 10 °C greater in 2021 compared to 2020. Further, lower mean soil moisture levels in 2021 could have affected photosynthetic performance. Our findings on F_V/F_M trends support our hypotheses with decreasing photosynthetic performance between the two years and across species but differ from previous research where plants that underwent a passive heating treatment with a mean air temperature increase of 0.5°C for two growing seasons displayed higher photosynthetic performance when compared to the control (Llorens et al. 2004).

Maximum soil temperature, maximum soil moisture, maximum air temperature, maximum surface temperature, the interactive effect of maximum soil temperature and maximum air temperature, and the interactive effect of maximum soil temperature and maximum surface temperature were the best local abiotic environmental condition predictors of F_V/F_M with

increasing F_V/F_M when temperatures and soil moisture increase (Figure 17). Our findings on the predictability power of various temperatures indicate that maximum surface temperature is the best predictor of F_V/F_M from the list of variables considered (Table 17). Flowering plants, especially seedlings, are near the soil surface where heating is intense leading to plant vulnerability (Marias et al. 2017a) likely affecting photosynthetic performance. As discussed above, known local abiotic environmental conditions can drive F_V/F_M trends and have the potential to be used as a tool to understand photosynthetic performance of plants. Our findings supported our hypotheses where we predicted decreasing F_V/F_M in response to increasing temperatures. This sparked further investigation into the relationships between mean F_V/F_M and the means for each best local abiotic environmental condition predictor. When the relationships of the means for each variable (i.e., maximum soil moisture, maximum soil temperature, maximum air temperature, maximum surface temperature) and mean F_V/F_M were considered, treatment and species effects become more apparent (Figures 18 & 19). The relationship between mean F_V/F_M and mean maximum air temperature was negative across all species indicating a relationship similar to what was anticipated with our hypothesis. However, there remained no significant treatment effect between the heating treatment and the control (Figure 18). When these relationships were assessed by species, there was a clear pattern of *Achillea* having the greatest mean photosynthetic performance relative to each of the abiotic predictors followed by *Geranium* and *Penstemon* (Figure 19). Evaluation of the means for each best local abiotic environmental condition predictor and mean F_V/F_M highlight underlying species-specific trends identified in our findings above.

Mean F_V/F_M was correlated with the flowering duration of *Achillea* with increasing flowering duration coinciding with decreasing mean F_V/F_M values. This suggests that *Achillea millefolium* is well adapted to heat stress (Mann et al. 2022) with longer flowering in response to reduced photosystem II operations. Further, this finding could indicate a tradeoff between photosynthetic performance and reproduction. *Achillea* plants may be flowering longer in an effort to increase reproduction as the growing season nears to an end. Mean F_V/F_M was correlated with the maximum floral display of *Geranium* with increasing maximum floral displays coinciding with decreasing mean F_V/F_M. Similar to *Achillea* flowering duration, *Geranium viscosissimum* may be well adapted to heat stress (Scheinost and Stannard 2010) by producing more flowers over time or as a tradeoff to increase reproduction in response to reduced photosystem II operations (i.e., reduced photosynthetic performance). Mean F_V/F_M and treatment had no effect on flower onset likely because F_V/F_M was not significantly affected by treatment for there to be an effect on flower onset.

It should be noted for all F_V/F_M results presented here, that even the lowest F_V/F_M values fall within the range of a healthy plant. We believe that this indicates that either the treatments did not induce enough stress on the plants or the aspect of physiology that was affected by the treatment was not measured in this study (i.e., gas exchange, stomatal conductance, etc.).

Our findings evaluating the effects of heating on flower onset, flowering duration, maximum floral display, mean F_V/F_M, and the relationships between them may not display the true effect of a warming climate on flowering plants and thereby flowering communities in the Rocky Mountain Region of the US. In the western USA, temperatures are projected to increase by 2–5 °C by 2100 (Halofsky et al. 2018, IPCC 2021). However, our passive heating structures

increased mean surface temperature by <1 °C throughout the duration of the study, with the control treatment experiencing greater temperatures and a range of temperatures compared to the heating treatment as seen in Table 19. The passive heating structures in the experimental study area, which is open and exposed, may have provided shade and insulation for the individual plants assigned to the heating treatment. Therefore, the treatment may not have been as intense as would have been needed to elicit the expected plant response. Further, increasing the length of the study beyond two years may have invoked a treatment response using these passive warming structures, like in previous work (Durney et al. 2022). However, there were clear species-specific responses. *Achillea* flowered longer, maintained greater photosynthetic performance overall and relative to increasing mean maximum soil moisture, mean maximum soil temperature, mean maximum surface temperature, and decreasing mean maximum air temperature. *Geranium* flowered longer, produced slightly more flowers in 2021 compared to 2020, followed *Achillea* in maintaining healthy photosynthetic performance overall and relative to increasing mean maximum soil moisture, mean maximum soil temperature, mean maximum surface temperature, and decreasing mean maximum air temperature. *Penstemon* experienced no effects on flower onset, flowering duration, maximum floral display, and had the lowest photosynthetic performance compared to *Achillea* and *Geranium* but remained within healthy photosynthetic performance. These findings indicate that *Achillea* and *Geranium* are robust and resilient species in the face of variable environmental conditions.

Even though this research did not detect the expected responses to heating on three prominent native flowering species found abundantly and broadly in the western USA, specifically the Rocky Mountain Region, species specific responses to environment were

detected. This highlights the potential of *Achillea millefolium* and *Geranium viscosissimum* to be used in conservation efforts associated with drought conditions. These two species experience long flowering durations, produce many flowers, are known to interact with pollinators (Glenny et al. 2022), and most importantly experience optimum photosynthetic performance in increasing drought conditions. Existing research concerning pollinator restoration strategies through seed mixes identified *Achillea millefolium* as a reliable floral resource in variable environmental conditions, while *Geranium viscosissimum* was not directly evaluated for seed mixes but experienced high bee visitation rates (Glenny et al. 2022). For these reasons we encourage investigation to include *Achillea millefolium* and *Geranium viscosissimum* in plant rehabilitation efforts, erosion control, and pollinator restoration strategies in the Rocky Mountain Region.

Table 19: The minimum, maximum, mean, and median values for soil temperature, soil moisture, air temperature, and surface temperature for the control and heating treatments. Values were derived from the HOBO temperature loggers and soil dataloggers located in the center of each plot. Air temperature was the same for control and heating plots since the 12 plots shared the same air sensor.

CONTROL	SOIL TEMPERATURE		SOIL MOISTURE		AIR TEMPERATURE		SURFACE TEMPERATURE	
	2020	*2021*	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*
MINIMUM	8.5	4.2	0.094	0.1	-3.79	-4.4	-2.84	-9.80
MAXIMUM	27.65	27.00	0.346	0.343	38.82	41.20	66.09	69.00
MEAN	19.07	18.13	0.232	0.207	17.26	17.61	22.94	20.38
MEDIAN	19.7	19.37	0.220	0.188	16.29	17.11	17.38	17.86
HEATING	SOIL TEMPERATURE		SOIL MOISTURE		AIR TEMPERATURE		SURFACE TEMPERATURE	
	2020	*2021*	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*
MINIMUM	5.65	2.3	0.147	0.2	-3.79	-4.4	-1.57	-7.53
MAXIMUM	26.4	25.60	0.401	0.392	38.82	41.20	59.30	59.47
MEAN	18.60	17.44	0.298	0.242	17.26	17.61	22.12	19.49
MEDIAN	19.28	18.45	0.310	0.233	16.29	17.11	17.95	18.62

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CHAPTER FIVE

WINGS THAT MAKE WAVES

Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 3

Author: Kim Moss

Contributions: co-authored grant, received funds for research, created all the graphics and videos, wrote and contributed to drafting text for publication, and compiled all the elements into an iBook

Co-Author: Diane Debinski

Contributions: wrote grant, received funds for research, helped develop iBook themes, featured in iBook content, and wrote and contributed to drafting text for publication

Co-Author: Simone Durney

Contributions: helped develop iBook themes, featured in iBook content, and wrote and contributed to drafting text for publication

Manuscript Information

Kim Moss, Diane Debinski, and Simone Durney

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☐ Officially submitted
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☒ Published

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Wings That Make *Waves*



A close look at *Parnassius clodius*, as a lens into changes occurring in the climate and biodiversity

Kim Moss
Diane Debinski
Simone Durney

CHAPTER

1

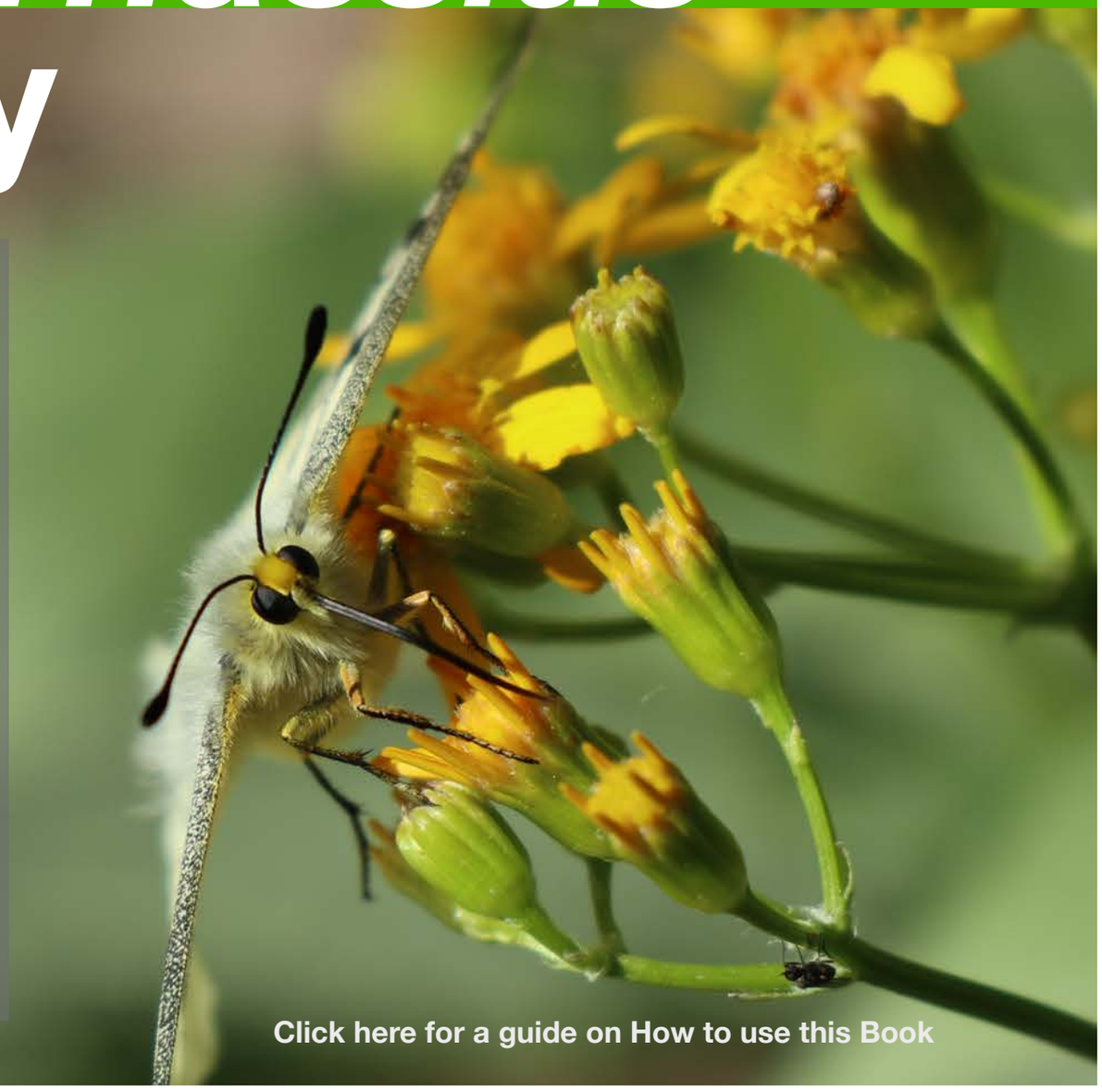
Wings That Make Waves

The *Parnassius* Story

Parnassius clodius is a butterfly that "makes waves" because of its link to changes occurring in the climate and **biodiversity** across the globe. Read on to discover how to identify and report sightings of this **species**, to learn how a blanket of snow protects it, and about the critical organisms that share its environment. By understanding *Parnassius,* you can make a difference in **conservation** and make waves too!

This iBook is free and you can download it from the Apple iBooks app to:

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- Learn how to become a **public scientist**
- See what is special about the alpine environment
- Learn how scientists study this important species



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Section 1

What Makes a Model Species?

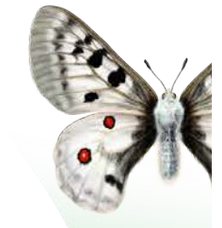
Imagine yourself immersed in nature on a sunny summer day, following a curious fuzzy butterfly as you hike among the mountain peaks in **Grand Teton National Park**. It's a lovely scene as you weave through a meadow, your eye catching the array of flower colors and greenery. The butterfly bobs in and out of the

plants, filling up on nectar, and zipping up into the air again to get “recharged” by the sun. You stop to simply take in the view and engrave it in your memory. What is this little light-colored butterfly that looks so at home among the alpine sunflowers and how did it come to live in such a beautiful and awe-inspiring environment?

Welcome to an amazing story about a little-known mountain butterfly called, *Parnassius clodius.* You’re about to discover why this species is quite a sight to see and is critical to understanding and protecting the mountain **habitat** you imagined above. In fact, *Parnassius* butterflies are not only linked to **alpine meadows.** They also provide a window into the **climate changes** occurring all over the world, making it a model species to study and monitor. Keep chasing this butterfly in the pages that follow to learn more.

The *Parnassius* butterfly is a medium-sized butterfly (wingspan of 2-2.5 inches or 50-62mm) that lives in **montane** (mountain) regions across the globe. *Parnassius* butterflies link the Greater Yellowstone **Ecosystem** of Montana and Wyoming in the United States to national parks and protected areas world-





Worldwide Presence

How do you say, *Parnassius clodius?*







wide. For example, these butterflies also live in the mountains of Canada, Europe, and Asia.

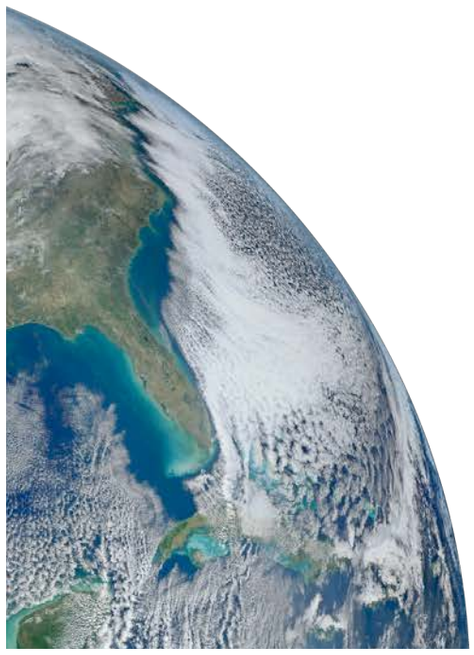
Parnassius are a high-**elevation** and high-**latitude** species, inhabiting unforested mountain meadow areas above the **treeline**. They are true mountain-dwellers and do not migrate south when the snow falls. After overwintering under the snow, their eggs hatch into caterpillars in the spring at the time of **snowmelt**.



However, spring in the Rocky Mountains is still very cold, and snow can occur until June! Therefore, these caterpillars are particularly vulnerable to cold temperatures when they emerge.



Parnassius clodius feeding on nectar from the Sulfur Buckwheat flower in the Greater Yellowstone Ecosystem (no audio).



Some of the most dramatic effects of **climate change** are happening at high elevations and high latitudes, so mountain meadow butterflies across the world are especially susceptible to habitat loss, **weather** events, and habitat modification.

The climate is getting warmer, seasonal patterns are changing, and plant communities are shifting in response to **climate changes**. As a result of these changes, as well as human-induced contamination, *Parnassius* butterflies are in decline, specifically in Europe. For that reason, it is important for conservationists, scientists, and the general public to become more aware of this species and to protect it from future decline.



Butterfly Conservation Biologist, Dr. Diane Debinski, on the importance of studying Parnassius.



PhD student, Simone Durney, sharing how Parnassius links to the ecosystem.



Ben Sisson, Butterfly Technician.

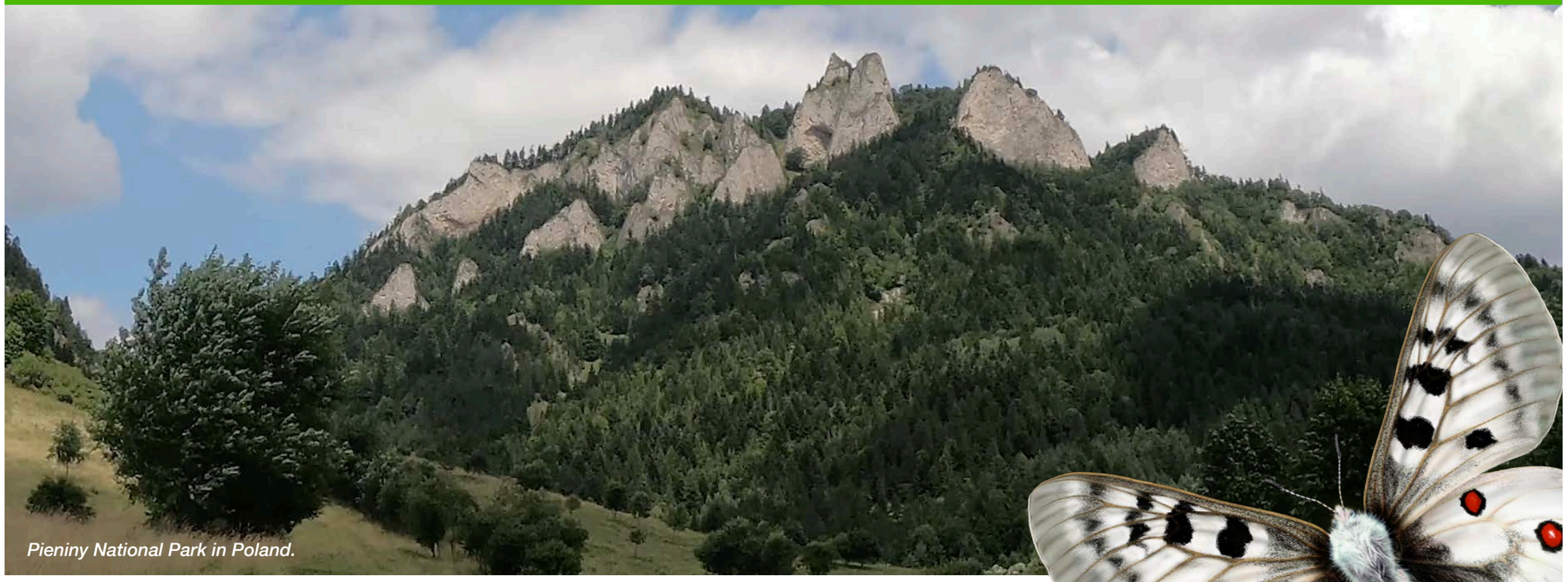
Seeing small things in a BIG way



Kim Moss, Scientific Illustrator and Instructional Designer.

Section 2

Why Does Monitoring Sister Species Matter?



Pieniny National Park in Poland.

P. apollo.

In Europe, a **sister species,** *Parnassius apollo,* a close relative to *Parnassius clodius,* is threatened with possible **extinction**. Note that these two butterflies have the same genus name *(Parnassius),* but different species names *(apollo vs. clodius).*



Butterfly Ecologist, Dr. Andrzej Kędziorski of the University of Silesia in Katowice, Poland, shown at Pieniny National Park.

Parnassius apollo, is listed in an international list of rare species called the "**Red Book**" under the category **"Near Threatened."** The Red Book is a list that is organized by a group called the **International Union for the Conservation of Nature (IUCN).**

In places like Poland, Finland, and Hungary, *Parnassius apollo* has become rare due to habitat loss and toxic metal contamination of the soil. To let people know how important it is to protect *Parnassius apollo,* it has become a symbol for Pieniny National Park in Poland—to promote awareness, they even have refrigerator magnets with images of the butterfly on them! To help increase the butterfly population in Poland, scientists are doing captive rearing of *Parnassius.* This means they are trying to raise caterpillars to the butterfly stage indoors so they can release the butterflies in the wild and help increase the **population** size.



Another sister species is *Parnassius smintheus* and it lives from New Mexico state, in the U.S. to the Yukon, in Canada. Unfortunately, this butterfly is also suffering from habitat loss. Over the past several decades, trees have started growing higher up on the sides of the mountains, making the forests larger, closing in on the meadows, which are now smaller.

There are many places across the globe where *Parnassius* are becoming rare. Thankfully, *Parnassius clodius* is not listed as endangered or threatened in the U.S. But given the vulnerability of its sister species, it is important to regularly survey populations of these butterflies and their habitats in places like the Tetons to make sure they can survive and potentially even thrive.

Given the rareness of sister species *Parnassius apollo* and *Parnassius smintheus,* it is important to survey and monitor the **distribution** (where they are located) and **abundance** (how many there are) of *Parnassius* butterflies in the Tetons. One way to do this is to enlist “public scientists,” people just like you, to report when and where they see *Parnassius* butterflies.

When hiking through Grand Teton National Park or places within the Greater Yellowstone Ecosystem, you can report sightings of the butterfly. You can note or report a sighting using your phone notes and camera or write down the date, time, and location to report later using a computer. We provide information about how you can do just that in this book, in Chapter 2, Section 4.



Butterfly Ecologist, Dr. Andrzej Kędziorski of the University of Silesia in Katowice, Poland, shown at shown at the University of Silesia discussing the status of Parnassius apollo in the Carpathian Range in comparison to Parnassius clodius in the Teton Range.

Section 3

Check Your Knowledge



Review 1.1

What is a sister species?

- **A.** A species that lives in the same region as the species of interest
- **B.** A species, at any location worldwide, that has the same genus name and is closely related to the species of interest
- **C.** The female version of a species

Check Answer

Do you think studying *Parnassius* can give us insight into other insect species?

Review 1.2

What threats are causing *Parnassius* butterflies to become rare in other countries?

- **A.** Loss of habitat
- **B.** Chemical contamination of habitat
- **C.** Trees moving into meadow habitats
- ✓ **D.** All of the above

Check Answer

Review 1.3

Why is it important to monitor *Parnassius clodius* in the U.S.?

- **A.** The U.S. population is currently large, so we would want to know if it starts to decline.
- **B.** *Parnassius* butterflies help us understand how other insects in the same ecosystem that are harder to study might be affected by environmental change.
- **C.** Some of the other *Parnassius* species worldwide are already rare.
- ✓ **D.** All of the above

Check Answer

CHAPTER
2

Wings That Make Waves

Finding *Parnassius*



If you were to imagine a flying polar bear, it might make you laugh to picture such a sight. But “flying polar bear” is the nickname butterfly biologist, Diane Debinski, gave *Parnassius clodius* because it is a small snow-colored butterfly with large black eyes and its body is incredibly furry!

Section 1

How Do You Spot a Flying Polar Bear?





The first lesson in identifying butterflies in mountains is to tune into **“Microfauna.”** It is all about noticing organisms of all sizes in your surroundings and fine-tuning your observation skills. “Micro” means small and “fauna” means animal.

To see butterflies and understand their habitat, you have to go to **alpine meadows** on a sunny day, slow down a bit, and open your eyes and ears to what is happening around you. Sometimes, you even need to crouch down to get eye-level with the mountain flowers to really see what is there. It’s a little bit like playing the

“Micro-Fauna” = Small Animal

game, “I spy” where you have to search carefully for details in your surroundings. In fact by doing this, you become a detective with a sense of inquiry to find clues based on your observations. In other words, you become a public scientist! Let’s give it a try. Imagine you are walking through an alpine meadow. Can you find the *Parnassius apollo* butterfly in the image below? (Hint: search in the middle of the image. Swipe across the image to see where the butterfly is hiding.)



So what do they look like? The unique polar bear-like "furry" appearance of *Parnassius clodius* comes from **"setae,"** which is "hair" in insect science-terms. On a *Parnassius* butterfly, there are lots of light-yellow setae on their head and body. As you can see from the pictures in this book, this medium-sized butterfly is indeed furry, but can you detect other features that make it unique? Describing the visual characteristics, or **morphology,** of an organism is really important to science and to identifying a butterfly in its habitat. Let's review the morphology of our little flying polar bear!

Notice that *Parnassius clodius* is a light color, similar to snow, and this color makes it blend right into the landscape of snow and rocks, which is a valuable trait! Why do you suppose that is valuable? Not only is *Parnassius* snow-colored, its wings are somewhat transparent (see-through) with some black markings and a few red spots. Notice the location and the shape of the red spots. Are they located on the **hindwings** (the set of wings toward the tail-end of the body), **forewings** (the wings toward the head), or both? Do they look the same on both front and back (ventral and dorsal) sides? Do you notice any other colored spots? How does the wing shape compare to other butterflies you have seen?



Parnassius clodius also has entirely black antennae, which helps distinguish it from other *Parnassius* species. This takes super keen observation to notice, but if you take your time, you can see this important feature. Practice your sharp focus by noticing small details around you. You are honing your skills as a butterfly biologist. Good work, detective!

Larger than the little "blue" butterflies commonly seen, but smaller than swallowtail, or monarch butterflies, *Parnassius clodius* is approximately 2-2.5 inches or 50-62 mm wide if you measured its wingspan. As we said, it also has big, dark, black eyes, so if you look at a *Parnassius* closely, it will look like it is looking right back at you!



And, just like you and me, not every butterfly within a species looks exactly the same. There is variation, or differences among individuals in a population. In fact, male and female *Parnassius* butterflies look slightly different. Scientists call this **“sexual dimorphism.”** For example, look at the **abdomens** of *Parnassius* in the videos below.

You can probably think of a few common examples of sexual dimorphism in animals you’ve seen. Consider the bright red male songbird, the northern cardinal, compared to the cinnamon brown female cardinal, or a male elk with its large size and antlers versus the smaller female elk without antlers. How do you think differences in morphology between males and females benefit a species? Consider how they behave differently and read on.

The females spend much of their time laying eggs. They don’t want to be seen by predators. Their dark and shiny bodies are less visible than the males, which have yellow fuzzy bodies.

Review the videos below and the illustrations on the next page. See if you can identify the differences in the male and female *Parnassius* butterflies in the pictures in this book. Consider what makes *Parnassius* an insect and see the next pages to find out more.



Parnassius clodius female.



Parnassius clodius male.

Parnassius clodius



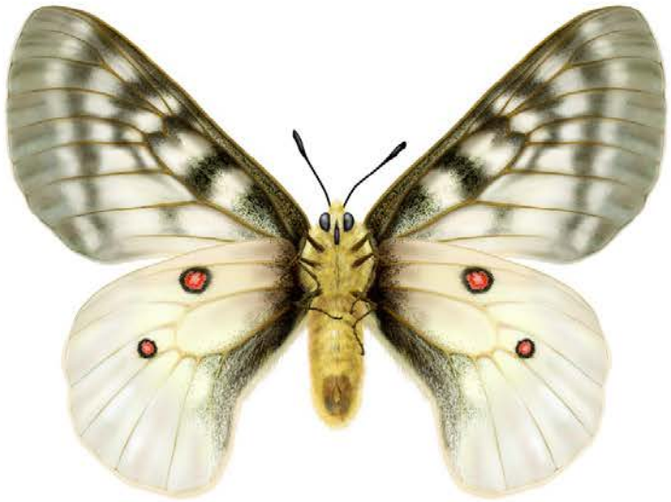
Female dorsal (back) view



Female ventral (front) view



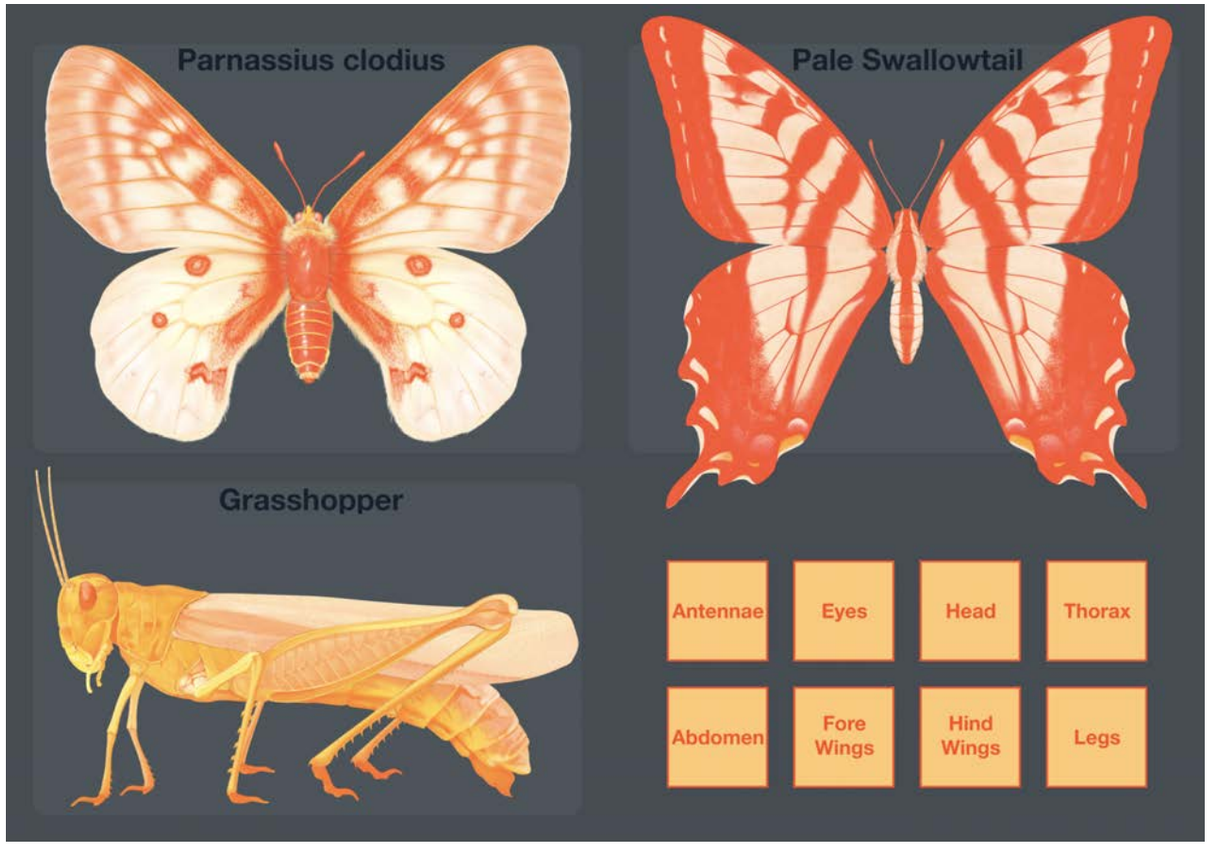
Male dorsal (back) view



Male ventral (front) view

Parnassius clodius is an insect. But how do we know it is an insect and how does it compare to other insects? Understanding these traits will help you identify butterflies as insects and notice the larger context, or group of organisms in which they fit.

Check out the Insect Body feature to the right to explore what *Parnassius* has in common with a grasshopper and swallowtail butterfly.



The Insect Body. Insects have bodies that include three main parts: the head, thorax, and abdomen. Tap on any of these insect's body parts to see how they relate to each other, or explore this by tapping the labels.

How is a butterfly similar to a grasshopper?

(Hint: check out the feature above.)

Check out these additional examples of sexual dimorphism. Can you spot the morphological differences between males and females? Swipe through the images below.



Male elk

Section 2

Field Guide: Where in the World is *Parnassius?*

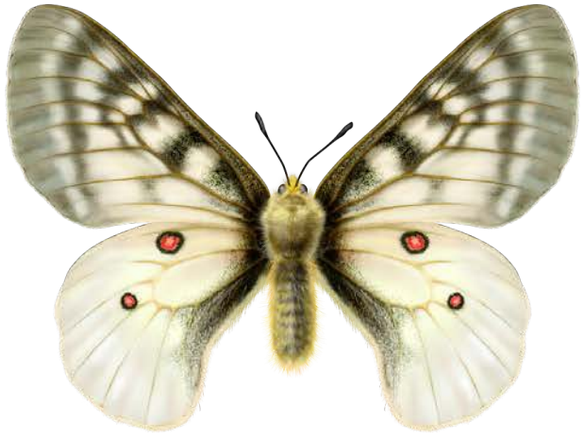
Parnassius mnemosyne

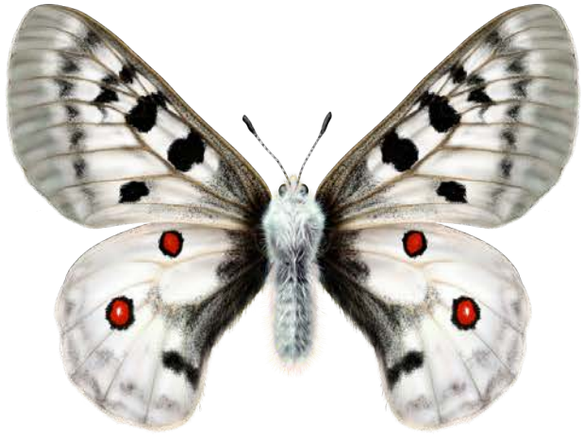




Parnassius smintheus

Parnassius clodius





Parnassius apollo

As mentioned earlier, *Parnassius (P.) clodius* has several sister species and in fact, there are three that we will discuss: *P. smintheus, P. apollo,* and *P. mnemosyne.*

P. smintheus also lives in the U.S. and has wings that are much more white in appearance and less transparent than *P. clodius.* Importantly, it has striped black and



Parnassius apollo.

white antennas rather than solid black and this is the easiest way to distinguish the two species if you get a close look.

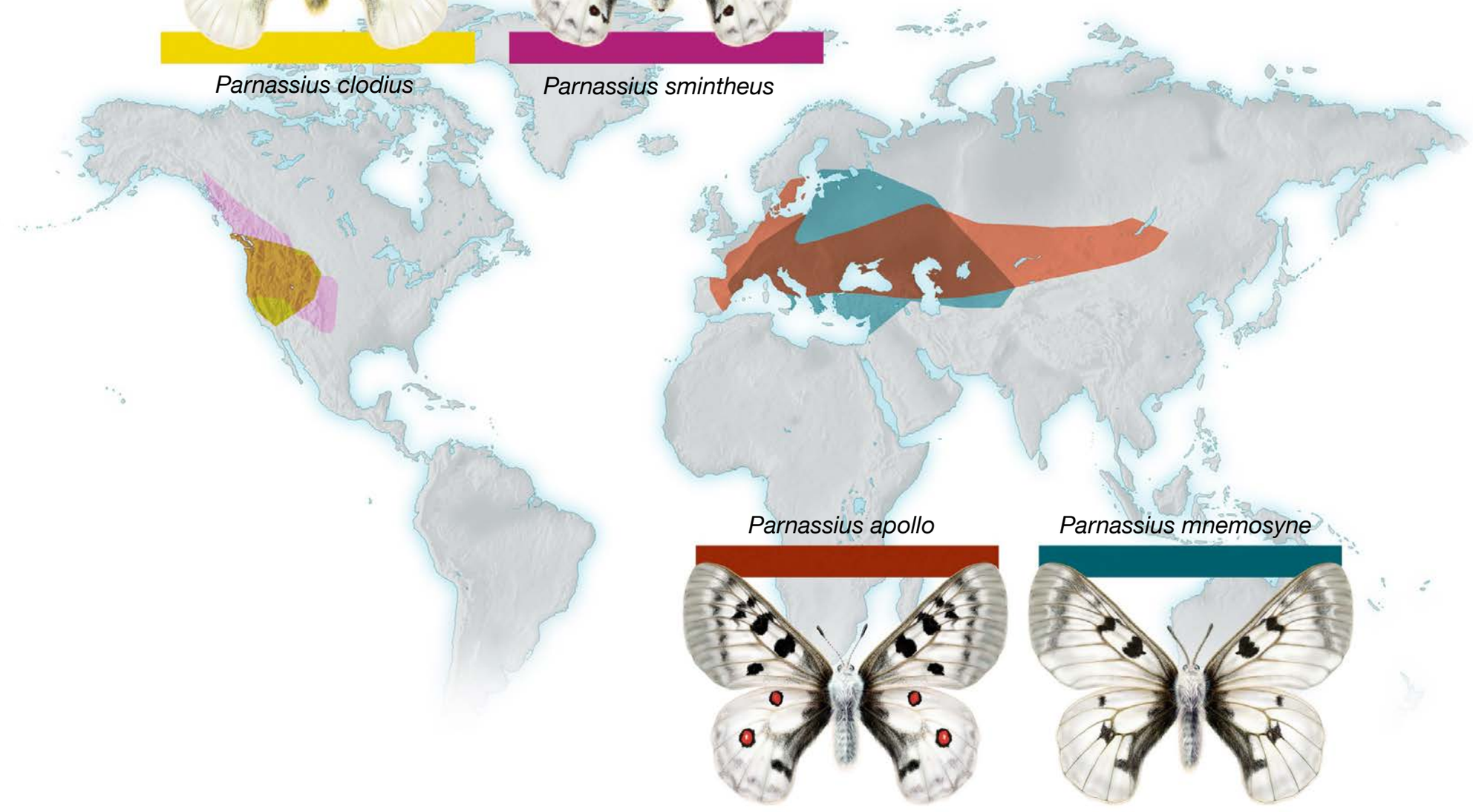
P. apollo also has slightly different black markings on the forewings, light-colored striped antennae, and bolder red dots on the hindwings. *P. mnemosyne* has the least amount of markings on its wings—there are no red dots and only a few dark spots on the hind- and forewings compared to the rest of the *Parnassius* group. Both *P. apollo* and *P. mnemosyne* live in Europe.

Study the pictures in this book to see if you can tell each *Parnassius* sister species apart. Do you think you would be able to recognize these butterflies in the field?



Parnassius clodius

Parnassius smintheus



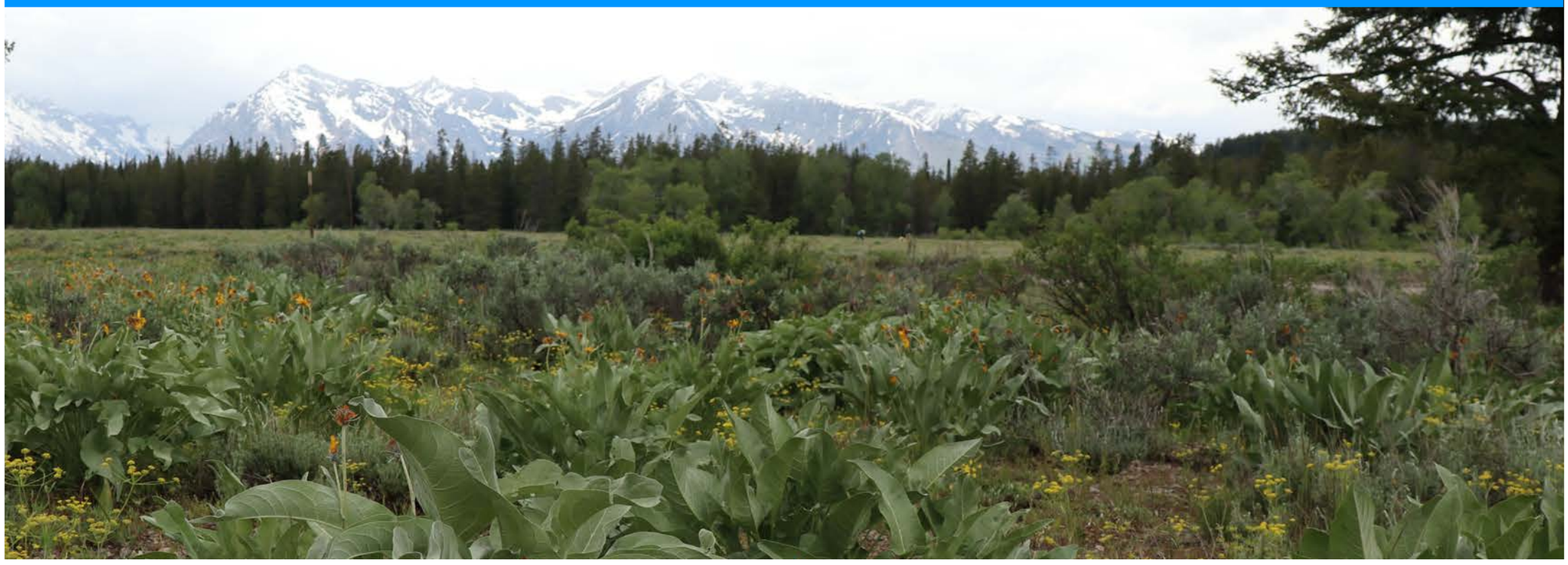
Parnassius apollo

Parnassius mnemosyne

Map Data Sources: https://www.butterfliesandmoths.org/species/Parnassius-clodius,
https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.121152/Parnassius_clodius

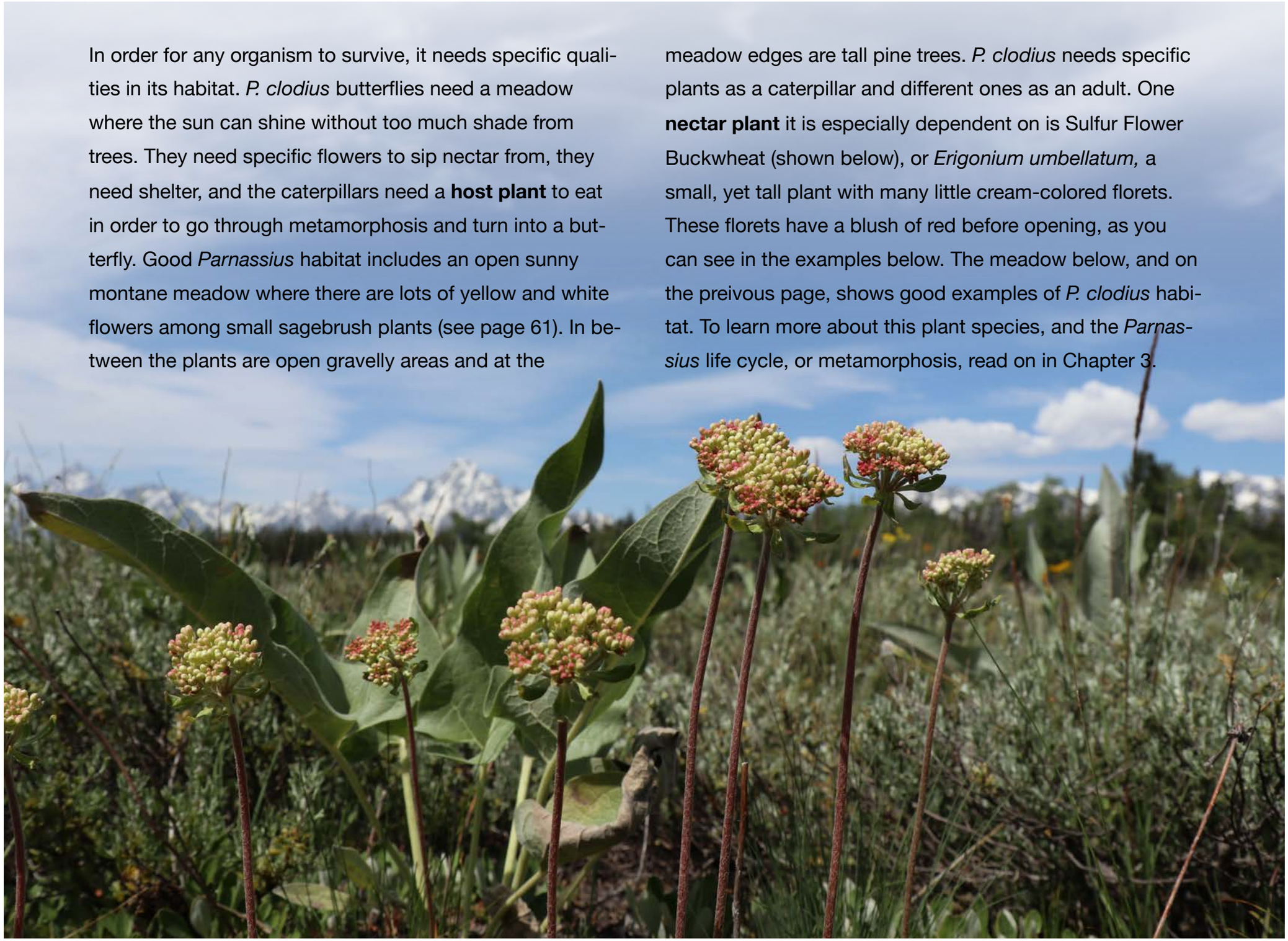
Section 3

Field Guide: Where Does *Parnassius clodius* Live?



If you have ever hiked in the mountains in the western U.S. and came across a lovely meadow with flowers, you may have been visiting the habitat of *Parnassius* butterflies. The butterflies can also be found up on top of mountains and along the habitat edges where the meadow meets the forest. Why do you think *Parnassius* butterflies live in **montane meadows**? How do you think they survive there?

In order for any organism to survive, it needs specific qualities in its habitat. *P. clodius* butterflies need a meadow where the sun can shine without too much shade from trees. They need specific flowers to sip nectar from, they need shelter, and the caterpillars need a **host plant** to eat in order to go through metamorphosis and turn into a butterfly. Good *Parnassius* habitat includes an open sunny montane meadow where there are lots of yellow and white flowers among small sagebrush plants (see page 61). In between the plants are open gravelly areas and at the meadow edges are tall pine trees. *P. clodius* needs specific plants as a caterpillar and different ones as an adult. One **nectar plant** it is especially dependent on is Sulfur Flower Buckwheat (shown below), or *Erigonium umbellatum,* a small, yet tall plant with many little cream-colored florets. These florets have a blush of red before opening, as you can see in the examples below. The meadow below, and on the preivous page, shows good examples of *P. clodius* habitat. To learn more about this plant species, and the *Parnassius* life cycle, or metamorphosis, read on in Chapter 3.



Section 4

What Do You Do When You See a *Parnassius* Butterfly?



So now that you know what our flying polar bear looks like and have learned about its habitat, wouldn't it be exciting to see one in the wild? If you plan on taking a hike in the mountains this summer, perhaps in a place like Grand Teton National Park, there's a chance you will see *Parnassius clodius.* In fact, you can download this book for free and use the pictures as a field guide on the trail.

But please note, if you see a butterfly in the mountains, take care not to hurt it or touch it. Instead, take a picture, and note your location and the weather conditions. Try to determine if it is indeed a *P. clodius* butterfly by referring to the pictures and descriptions above.

You can help scientists track butterflies by uploading your photograph and reporting the location to **eButterfly**, a website collecting this kind of data. Then you will be a public scientist, helping to take care of and monitor these important butterflies!

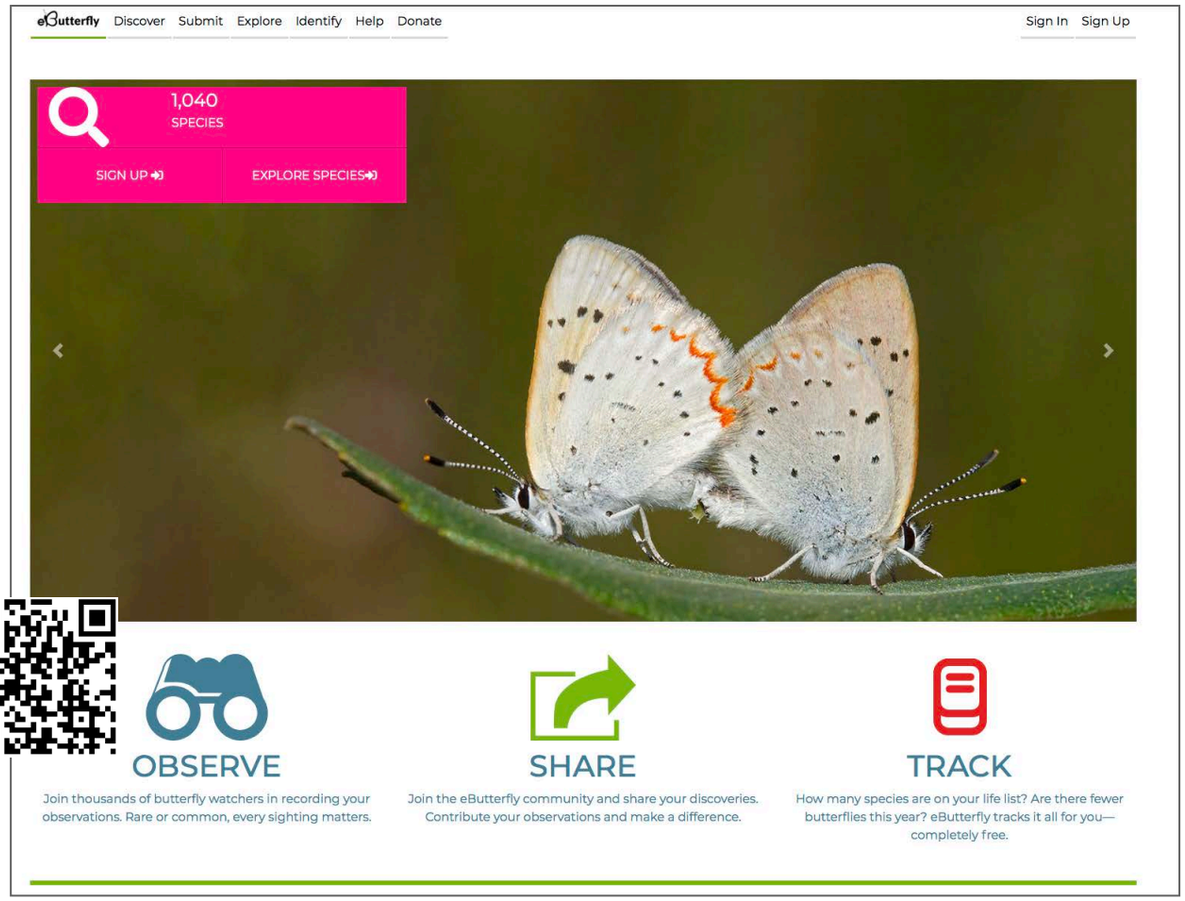


The app, ***iNaturalist,*** has great tips for taking photographs of organisms in the wild with lots of helpful videos, found here.



To report a *Parnassius* sighting to eButterfly, go to http://www.e-butterfly.org/, sign up or sign in, and click on Submit. You will want to note the date and your location on a map, or GPS coordinates, and also observe and record the weather. Reporting any *Parnassius* butterflies you see can really help scientists understand where they live and how many there are. By doing this, you are using your keen butterfly observation skills and providing important data! If you don't have internet connection when you see a butterfly, do not fret. Simply record the information above and enter it later when you can connect. See how to do this here.

1. Observe a butterfly in the field.
2. Take a picture.
3. Collect data: write down your location, the weather conditions, and notes about what the butterfly looks like (for example, how many spots it has).
4. Log on to e-butterfly.org and report your sighting and data: use the QR code at right.
5. What if you don't know the butterfly species? It's okay, check out this helpful video here.



Images used with permission from eButterfly. © eButterfly.

Section 5

What Are Waystations?



Butterfly “Waystations" are habitat plots where people grow specific plant species that butterflies need to survive. When butterflies are in the caterpillar stage, they use a “host plant” that they eat to provide nourishment. When butterflies are adults they need nectar plants for energy.

A Station With a Way



Montana State University Pollinator Garden.

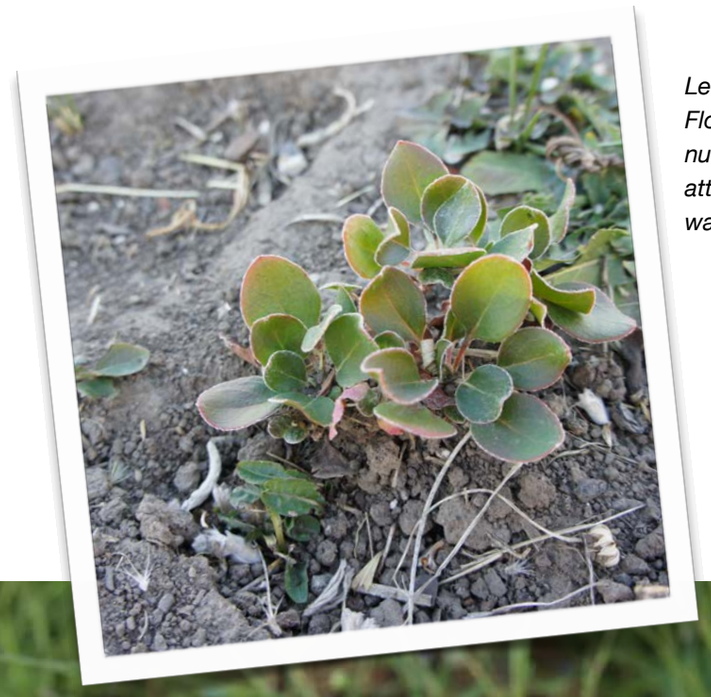


Parnassius clodius uses a plant called Steer's Head *(Dicentra uniflora.* See page 45) as a host plant. That means that it eats the leaves of this plant when it is a caterpillar. It sips nectar from nectar plants such as Sulphur Flower Buckwheat *(Eriogonum umbellatum.* See page 26) when it is a butterfly.

The butterfly waystation approach has been very effectively used for monarch butterflies across the U.S. and many people have planted milkweed in their backyards for monarch butterflies.

Parnassius scientists are testing out ways to grow the nectar plants for *P. clodius* and then plant these flowers in a **pollinator** garden on the

campus of Montana State University to create a Butterfly Waystation in the Bozeman, MT region. The host plants and nectar plants that *Parnassius clodius* use are not as available through commercial greenhouses, but some native plant nurseries sell *Eriogonum umbellatum.* There are also other plants such as yarrow *(Achillea millefolium),* sticky geranium *(Geranium viscosissimum),* and groundsel (Senecio spp.) that *P. clodius* can use as a nectar source (see also the Section in Chapter 3 on food and **foraging**). These can also be planted in **Butterfly Waystations** for *Parnassius.* The health and survival of these plants are closely linked to the survival of *P. clodius* so waystations for this butterfly are worth testing. Who knows, maybe you will meet our flying polar bear butterfly in a garden on a college campus someday!



Left: sprouts of Sulphur-Flower Buckwheat (Eriogonum umbellatum) as the first attempt to create a butterfly waystation for P. clodius!









Pollinator Garden and Monarch Waystation images from Neal Smith Wildlife Refuge in Iowa.



Section 6

Check Your Knowledge

Review 2.1

Pretend you spot a butterfly in the mountains. What features would you focus on and record?

- **A.** Size of the butterfly (small, medium, or large)
- **B.** Predominant color of the wings, both dorsally and ventrally
- **C.** Secondary colors
- **D.** Any important spotting patterns - number of spots, color of spots, and location on the butterfly
- ✓ **E.** All of the above

Check Answer

Review 2.2

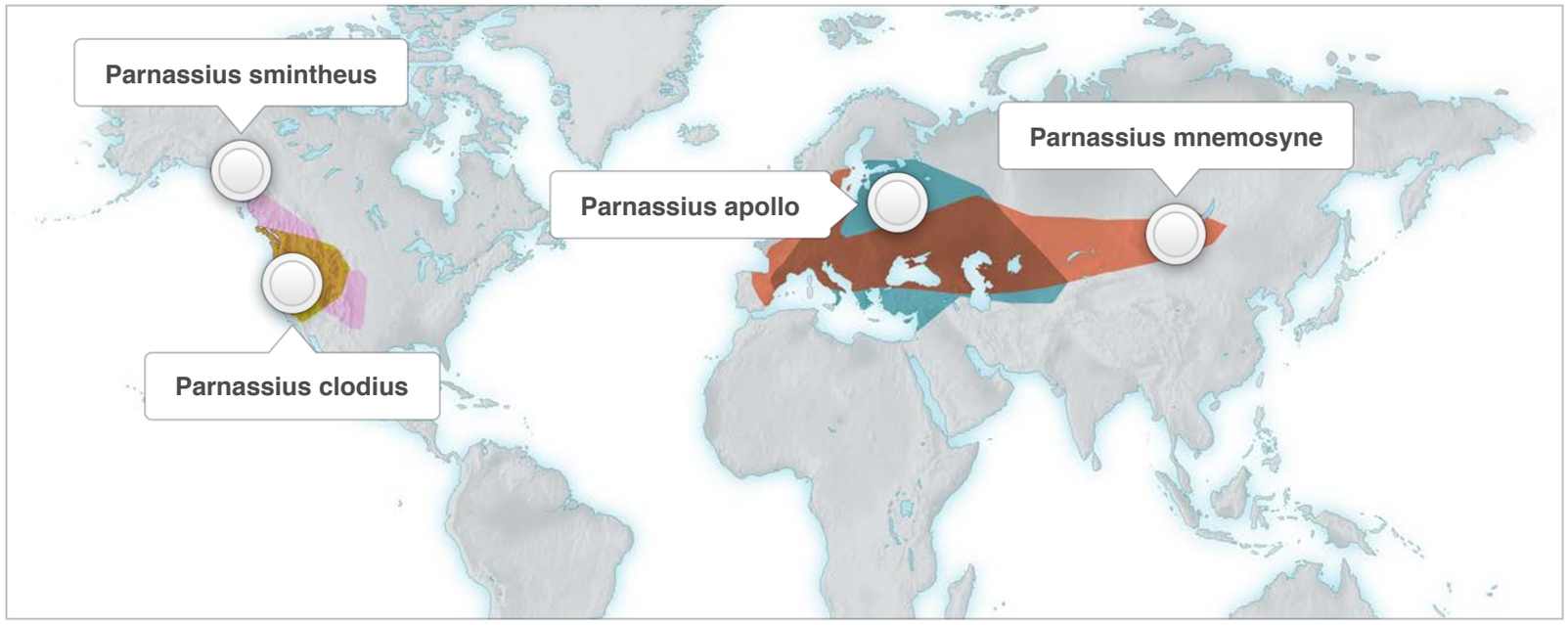
Which of these images shows *Parnassius* habitat?



Check Answer

Review 2.3

Match each *Parnassius* species to its range on the map. With your finger, drag and drop the labels to a colored area on the map.



Parnassius clodius | Parnassius apollo | Parnassius mnemosyne | Parnassius smintheus

Check Answer

What Does it Mean to be a Public Scientist?

A public scientist is a person who voluntarily collects data on a scientific topic and then reports that information to a person or computer website that collects that kind of data.

For example, if you are walking through a national park or even through your local park and you make note of all of the butterflies that you saw flying on a particular day, you could report those butterfly observations to the website called eButterfly, mentioned in Section 4 of this Chapter. You can take a photo or make notes about the date, time, and location of these observations to report later using a computer.



CHAPTER
3

Wings That Make Waves

Parnassius in the Wild



Many animals migrate south in the winter, like the well-known monarch butterfly. But most butterflies do not migrate and thus live in the same place all year. In order for *P. clodius* to survive the winter, it has to mate in the summer and the female must lay eggs that overwinter in the mountains under the snow. Did you ever think of snow as a cozy warm wintertime blanket? You would if you were a tiny *P. clodius* egg!

Section 1

How is The *Parnassius* Lifecycle Unique?



Parnassius Life Cycle



Males are busy looking for females to mate with.

Parnassius clodius pairs mate. Females only mate once!





The Females lay their eggs right after mating.



Caterpillars depend on *Dicentra uniflora* for food. This plant comes out early in the spring for a short period of time.

Caterpillars form a chrysalis. After a few weeks, they turn into an adult butterfly, ready to fly!





Caterpillars hatch from eggs in the spring after snow melt.

June July

This is the main flight season for *Parnassius clodius.*

August September October November December January February March

Autumn & Winter

Temperatures cool early in the mountains. *Parnassius clodius* eggs stay put and overwinter, protected under the snow.

April May

It is critical that the timing of the top two spring events (egg hatching and *Dicentra uniflora* emergence) overlap, or *Parnassius* will not have a plant to feed on.



A male Parnassius clodius looking for a female.

The **lifecycle** of *P. clodius* begins in late June and July when males are flying around in search of females to mate with. Males butterflies emerge first and females come out shortly afterwards. Once a male finds a female, by seeing or smelling her, the male begins a courtship ritual to attract the female. View the searching male *P. clodius* in the video above.



If the female is willing to mate with that male, the two butterflies will join together (couple). The male will attach himself to the female by curling his abdomen under and clasping to the female's abdomen while they are both sitting on a plant or on the ground. The male will then deposit his sperm within the female to fertilize her eggs.

Once complete, the male will make a structure out of a thick protein-like material on the end of the female's abdomen, called a **sphragis**, to prevent other males from mating with her. This coupling process happens over the course of a few hours.



Parnassius coupling. Swipe through the images, or tap the arrows below, to view the gallery.

Once the two butterflies decouple, the female will fly off in search of areas to lay her newly fertilized eggs—often on the undersides of arrowleaf balsamroot leaves or on stems of sagebrush.

Adult *Parnassius* butterflies only live for a few months in the summer. By the end of July, all the *Parnassius* eggs have been laid in the meadow, and the adult male and female butterflies will have died. Between October and March, the meadows where these butterflies live are covered with snow and under that snow are the eggs laid by the female butterflies.



Female with fully-formed sphragis—she will not mate again. Swipe through the images, or tap the arrows below, to view the gallery.



Tap the label to see two *Parnassius* eggs up close!

The snow acts as a protective layer, shielding the eggs from harsh cold



during the winter months. By April and May, the snow begins to melt and the eggs hatch into caterpillars. Like most caterpillars, *Parnassius* caterpillars are hungry, so they spend most of their time in search of their **host plant,** Steer's Head *(Dicentra uniflora),* to feed on. Towards the end of May, the *Parnassius* caterpillars form a chrysalis and they stay in this stage for a few weeks before they emerge as a butterfly. Male *Parnassius* butterflies emerge in the middle of June shortly followed by female butterflies, and the life cycle begins again.





Steer's Head, Dicentra uniflora. Parnassius caterpillars search for this host plant once the snow melts in the spring.

Section 2

What does *Parnassius* Eat?

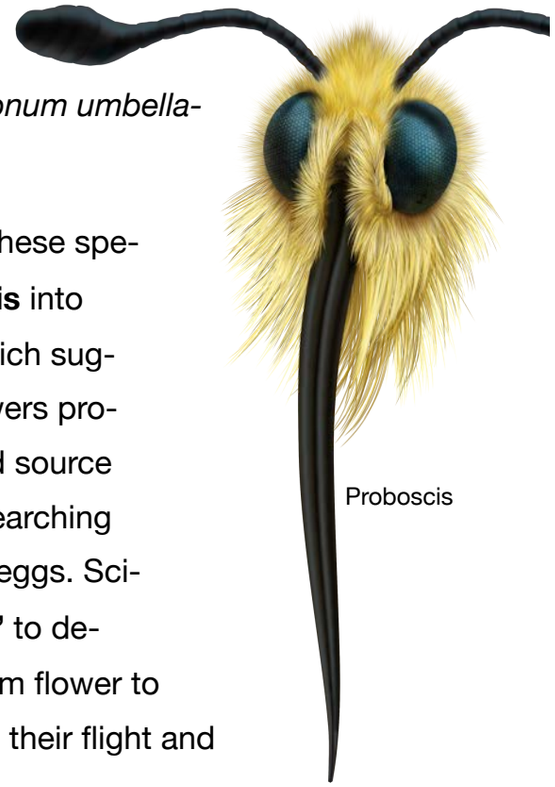


Parnassius are picky eaters, especially in the caterpillar stage: they are **specialists** and eat only one type of plant! (Butterflies that are **generalists** can eat many types of plants.) As a caterpillar, *Parnassius* only feed on the Steer's Head plant *(Dicentra uniflora,* shown above). Steer's Head is a short-lived rare plant that flowers early



in spring. It is often surrounded by patches of snow and prefers disturbed gravelly soils in montane and **subalpine** regions. It is unclear how these caterpillars find Steer's Head to feed on, but it is possible that they smell it out—however, that part of *Parnassius* natural history is yet to be fully understood!

Once *Parnassius* transform into a butterfly, they feed on plants that are flowering in June and July. However, they do not feed on all flowering plants during that time frame. Instead they feed on certain plants that are the right color and provide a nice landing platform for them to rest on as they sip nectar. This narrows the *Parnassius'* food or **nectar source** options down to only a small number of flowering plants species, including Arrowleaf Balsamroot *(Balsamorhiza sagittata)* and Sulphur Flower Buckwheat *(Eriogonum umbellatum)* as well as some others.



Parnassius butterflies feed on these species by inserting their **proboscis** into the flower and sucking up the rich sugary liquid nectar that these flowers provide. This nectar is a great food source for *Parnassius* while they are searching for a mate or for a place to lay eggs. Scientists use the term **"foraging"** to describe how butterflies move from flower to flower feeding on nectar to fuel their flight and prepare for reproduction.



Butterfly landing pads.

Finding landing pads



Parnassius feeding on Sulfur Flower Buckwheat, Eriogonum umbellatum.





Arrowleaf Balsamroot, shown above and above right, is a yellow sunflower that blooms in early spring among montane meadows. This flower is a valuable early nectar source for *Parnassius,* however, because these flowers bloom early in the spring, the butterflies only get to forage on them as the flowers are at the tail end of their flowering period.

Sulphur Flower Buckwheat, shown at left, is the main nectar source for the *Parnassius* butterfly in Grand Teton National Park. It begins to bloom when the butterflies emerge and flowers throughout the time that *Parnassius* butterflies are forag-



Parnassius feeding on Sulphur Flower Buckwheat.

ing and reproducing. This buckwheat species forms a mat of leaves with numerous cream flowers full of rich nectar among these mountainous meadows.

Some other nectar sources include groundsel (Senecio spp.), sticky geranium *(Geranium viscosissimum),* western giant hyssop *(Agastache urticifolia),* dogbane *(Apocynum androsaemifolium),* snowbrush *(Ceanothus velutinus),* spirea *(Spirea betufolia),* common snowberry *(Symphoricarpos albus),* which were identified by Kim Szcodronski, another *Parnassius* butterfly researcher.

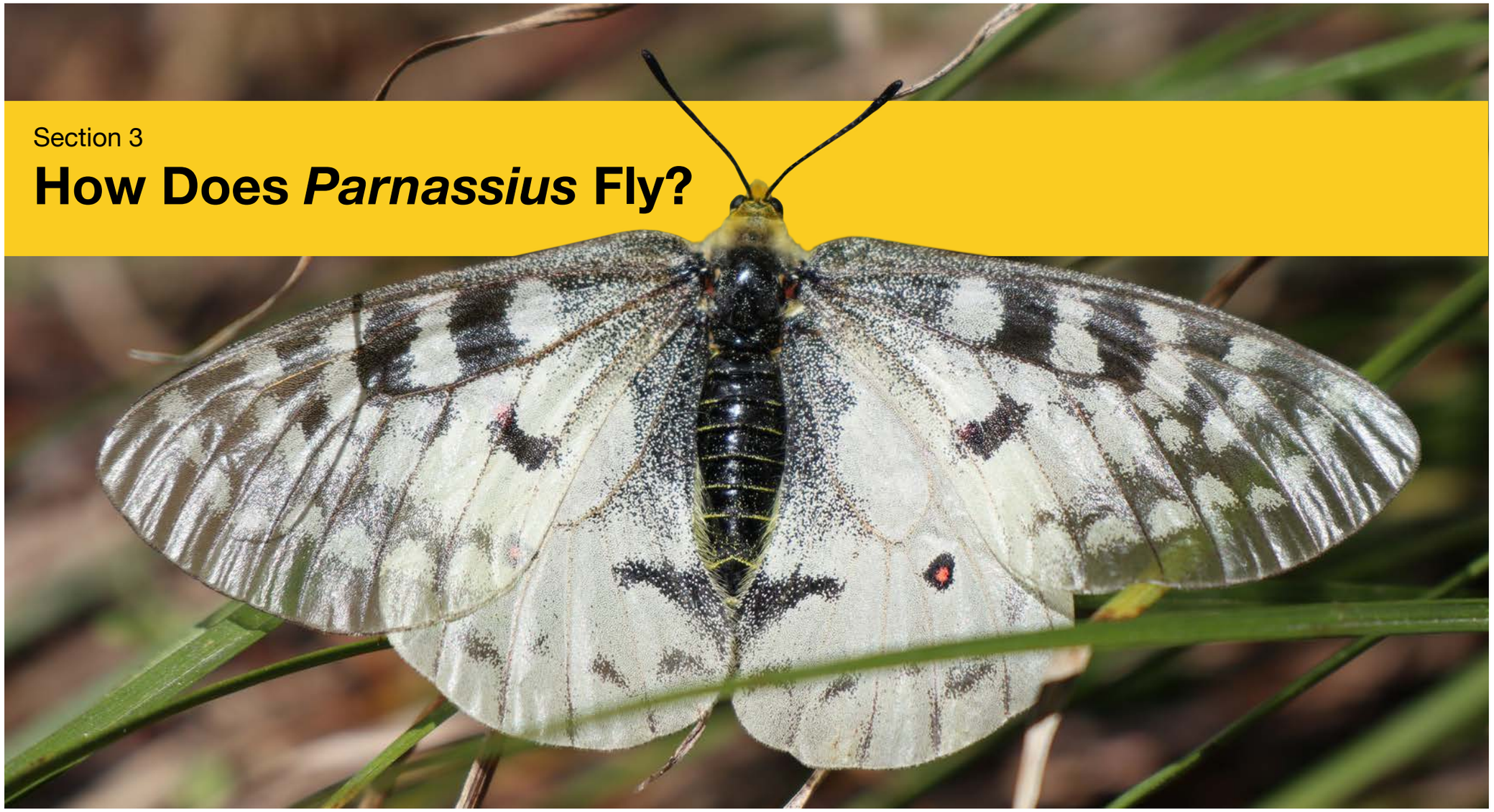
It is possible that the **"synchrony"** (two things happening at the same time) between when these flowers bloom and when the *Parnassius* butterfly is foraging could be affected by climate change. If the flowers respond to one environmental cue such as timing of snowmelt, and the **butterfly emergence** responds to another cue such as temperature, the synchrony between these two species could go away. This could present a threat to the butterfly because it might have to find another less nutritious plant from which to get nectar. It is important to continue surveying these butterflies over time to see what they are feeding on so that we can understand how a changing climate may affect this relationship.



Parnassius sipping nectar from groundsel (Senecio spp.).

Section 3

How Does *Parnassius* Fly?



Parnassius butterflies are solar-powered. This means they cannot generate heat from within their bodies and need to warm up from the sun before they can fly (see page 59). As mentioned earlier, they have a wingspan of about 2.5 inches or 6 centimeters, roughly the size of a car key. Because of the shape and size of their wings, they fly in a smooth rhythmic way. When you see a *Parnassius* flying, they appear to be floating along, gently

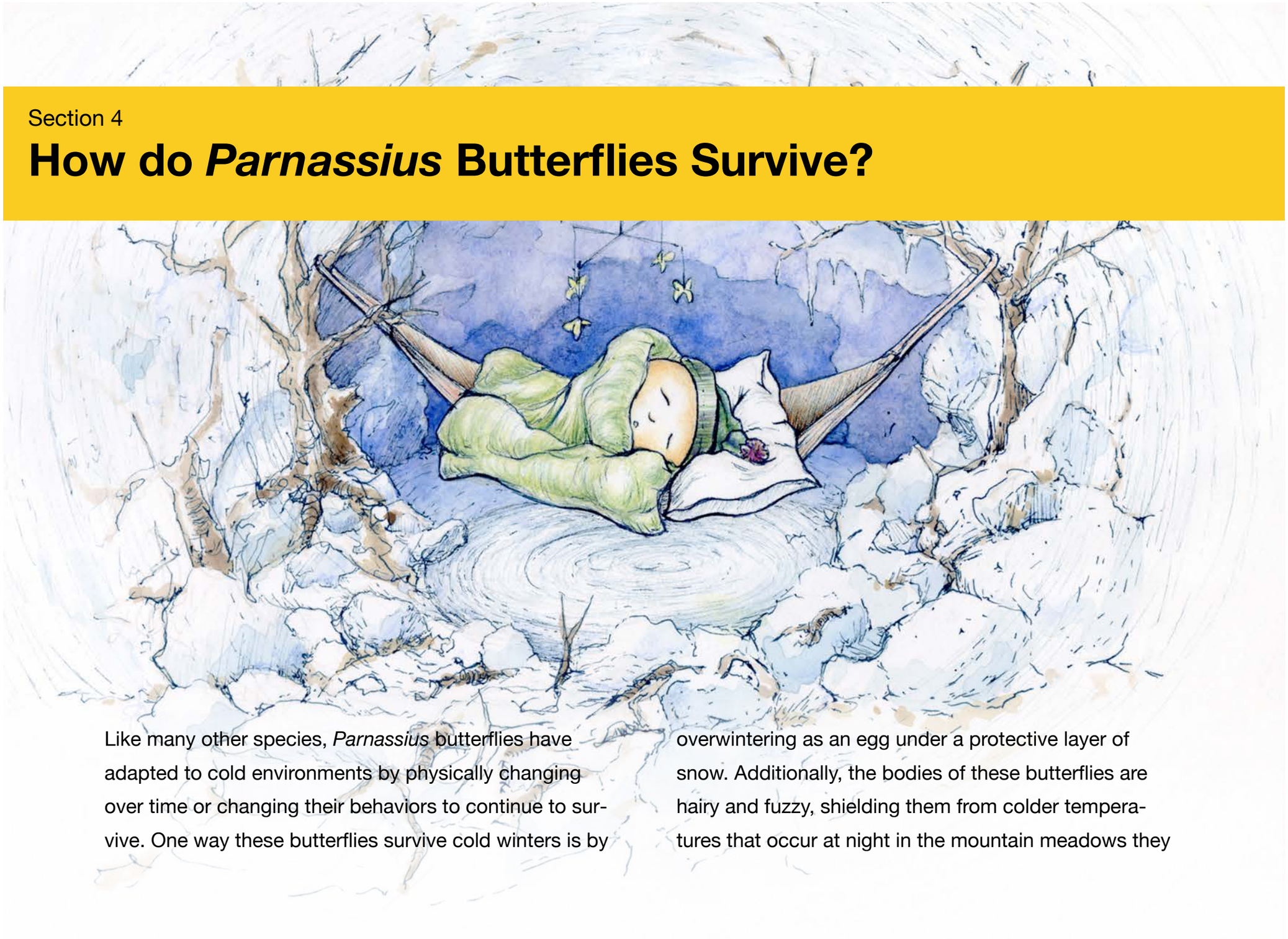


dipping down between sagebrush and then floating back up above the meadow plants. This flight pattern is often seen among male butterflies searching for emerging female butterflies to mate with. Butterflies also chase one another, darting up and down and side-to-side. This can be a way of a male courting a female, but this behavior can also happen between different species of butterflies as a way of figuring out whether they are the same species or not.


Solar-Powered,
Dynamic
Wing Shapes!

Section 4

How do *Parnassius* Butterflies Survive?



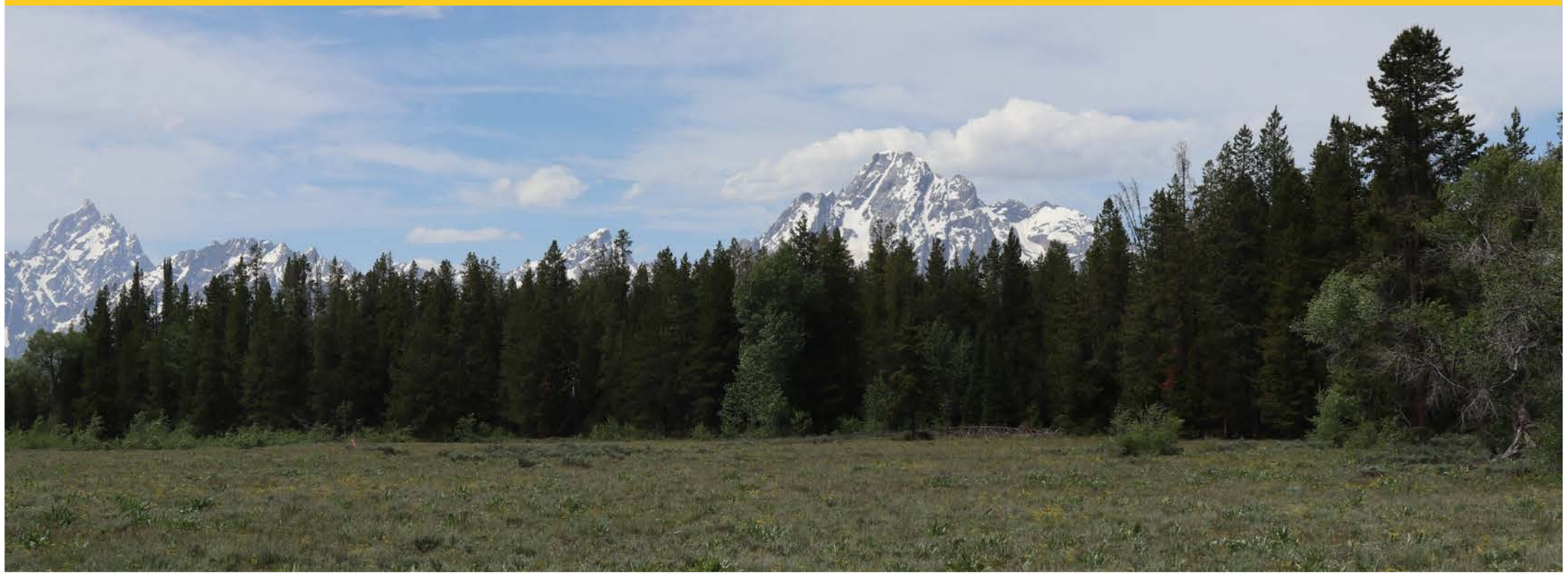
Like many other species, *Parnassius* butterflies have adapted to cold environments by physically changing over time or changing their behaviors to continue to survive. One way these butterflies survive cold winters is by overwintering as an egg under a protective layer of snow. Additionally, the bodies of these butterflies are hairy and fuzzy, shielding them from colder temperatures that occur at night in the mountain meadows they



are found. Also, female *Parnassius* butterflies have adapted their behavior to prevent being eaten by birds by not flying around as often as males and sitting still to blend in with their environment. And, you already know that they are similar in color to the snow and the light-colored rocky ground! Good camouflage keeps predators away.

Section 5

How Does *Parnassius* Use The Trees?



When male *Parnassius* butterflies begin flying in late June, they usually stay close to good habitat containing all the flowers they need. Once the female *Parnassius* butterflies begin flying in early July, the distances these butterflies move begins to increase as they mate and the females search for host plants to lay their eggs near and nectar plants to feed on. *Parnassius* butterflies often use the forest edge to move around. It is not exactly



known why they do this, but it is suspected that they use the edge as protection from wind and for cover. In open meadows these butterflies can fly longer distances than along a forest edge, averaging 202 meters or roughly the length of two football fields. *Parnassius* butterfly biologist, Julia Auckland, figured this out by using **mark-recapture surveys**. If you follow a *Parnassius* butterfly, you may see it fly from a meadow directly into a forest and seem to disappear. This may be a way to avoid predation by birds.

Section 6

Check Your Knowledge

Review 3.1

Review the pictures in the gallery at right. What are 2 nectar sources for the *Parnassius clodius* butterfly?

- **A.** Leopard Lilly and Sagebrush
- **B.** Milkweed and Black Eyed Susan
- **C.** Sagebrush and Purple Prairie Clover
- ✓ **D.** Sulfur-flower buckwheat and Arrow-leaf Balsamroot

Check Answer

Gallery 3.1 Which plants are nectar sources for the *Parnassius clodius* butterfly? Review the photos below by swiping through the image gallery, or use the arrows at the bottom. Select your answers in the panel to the left.



Sagebrush, *Artemiscia arbuscula*

Review 3.2

Name one way that *Parnassius* butterflies withstand the cold.

- **A.** The eggs can freeze
- **B.** The caterpillars can freeze
- ✓ **C.** The eggs spend winter under the snow
- **D.** The butterflies migrate to Mexico

Check Answer

Review 3.3

Why is a host plant important to a butterfly?

- **A.** It provides nectar
- ✓ **B.** It is the plant that the caterpillar eats and needs for nutrition
- **C.** It provides shelter
- **D.** It provides a landing pad for the butterfly

Check Answer

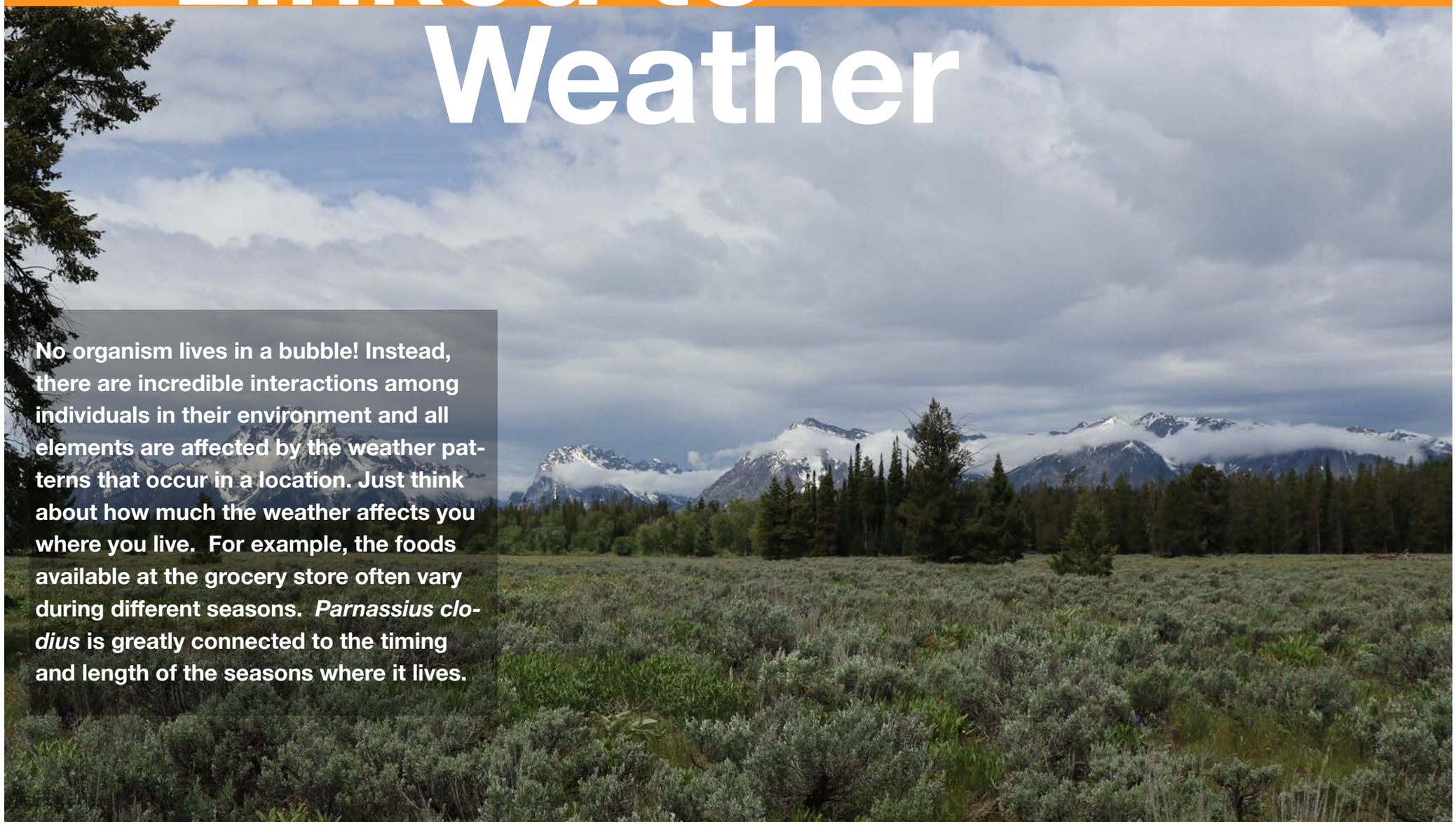
What makes *Parnassius* a specialist?

(Hint: see Section 2.)

CHAPTER
4

Wings That Make Waves

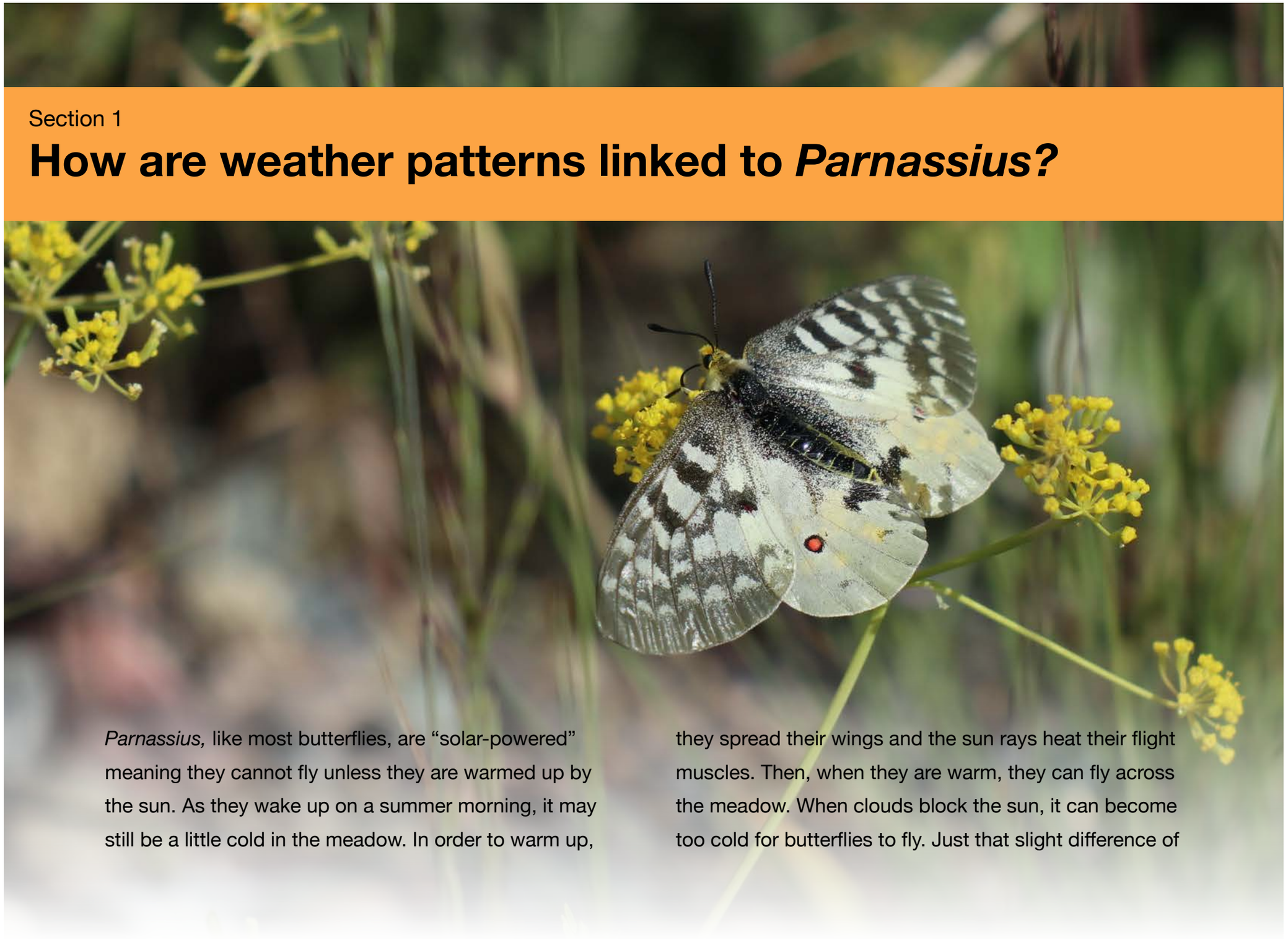
Linked to Weather



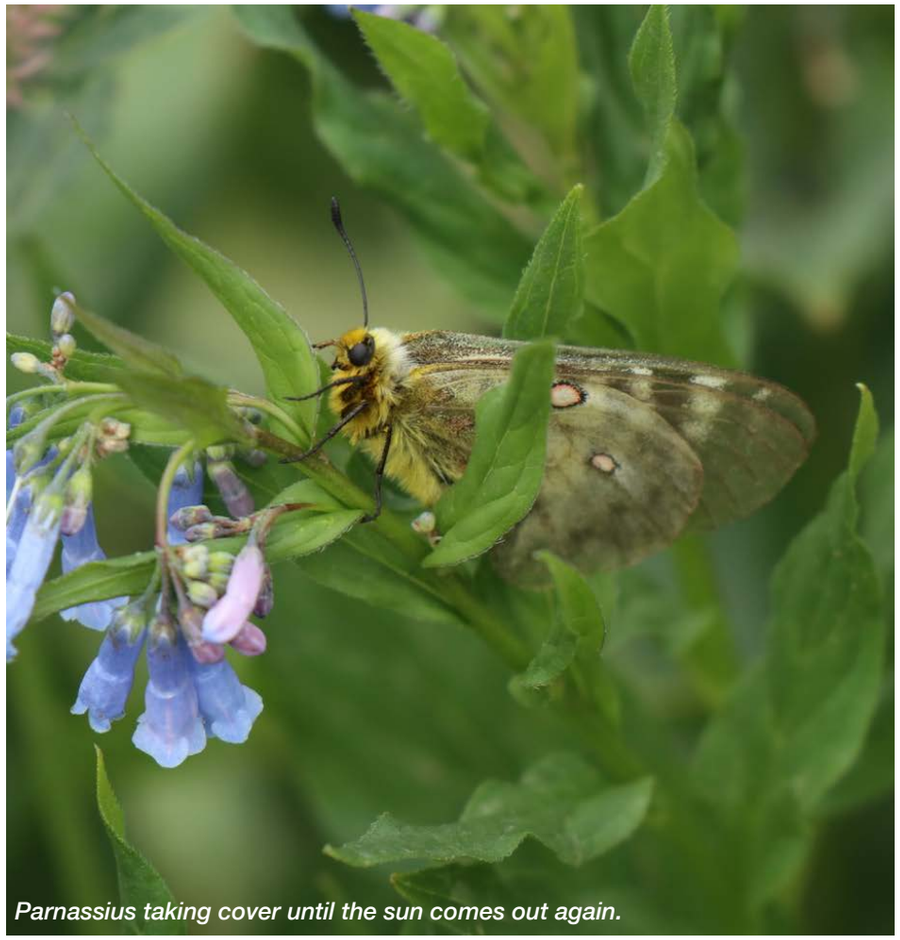
No organism lives in a bubble! Instead, there are incredible interactions among individuals in their environment and all elements are affected by the weather patterns that occur in a location. Just think about how much the weather affects you where you live. For example, the foods available at the grocery store often vary during different seasons. *Parnassius clodius* is greatly connected to the timing and length of the seasons where it lives.

Section 1

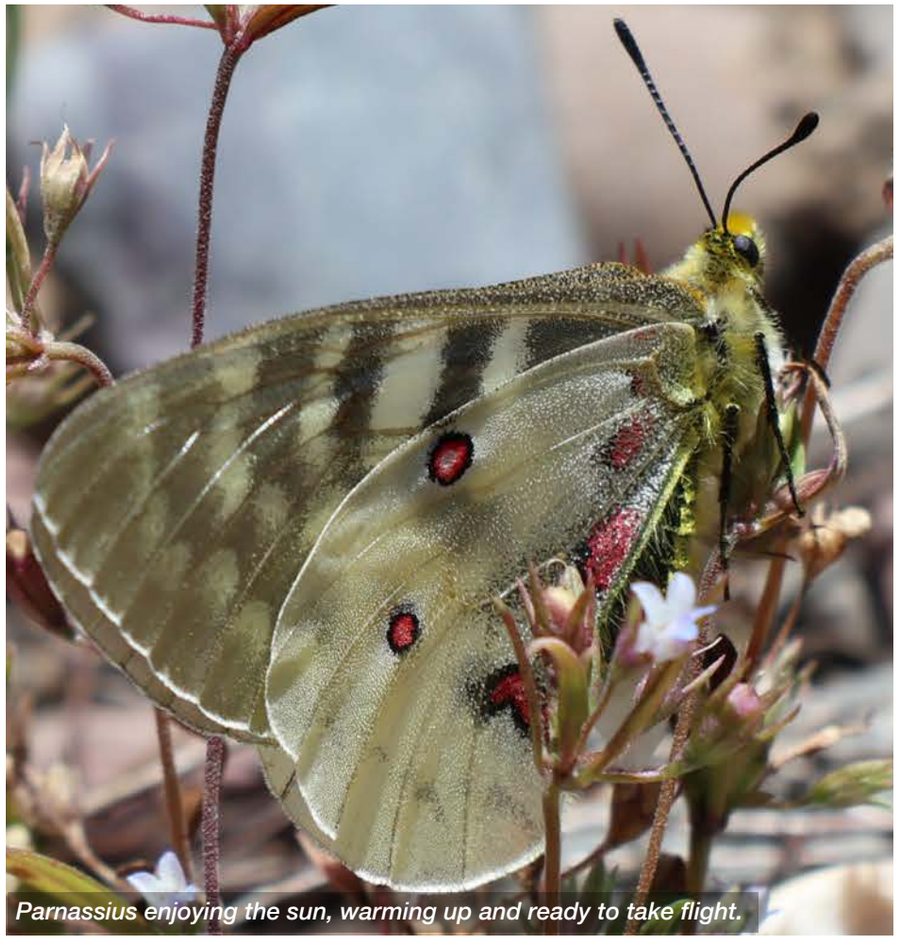
How are weather patterns linked to *Parnassius?*



Parnassius, like most butterflies, are “solar-powered” meaning they cannot fly unless they are warmed up by the sun. As they wake up on a summer morning, it may still be a little cold in the meadow. In order to warm up, they spread their wings and the sun rays heat their flight muscles. Then, when they are warm, they can fly across the meadow. When clouds block the sun, it can become too cold for butterflies to fly. Just that slight difference of



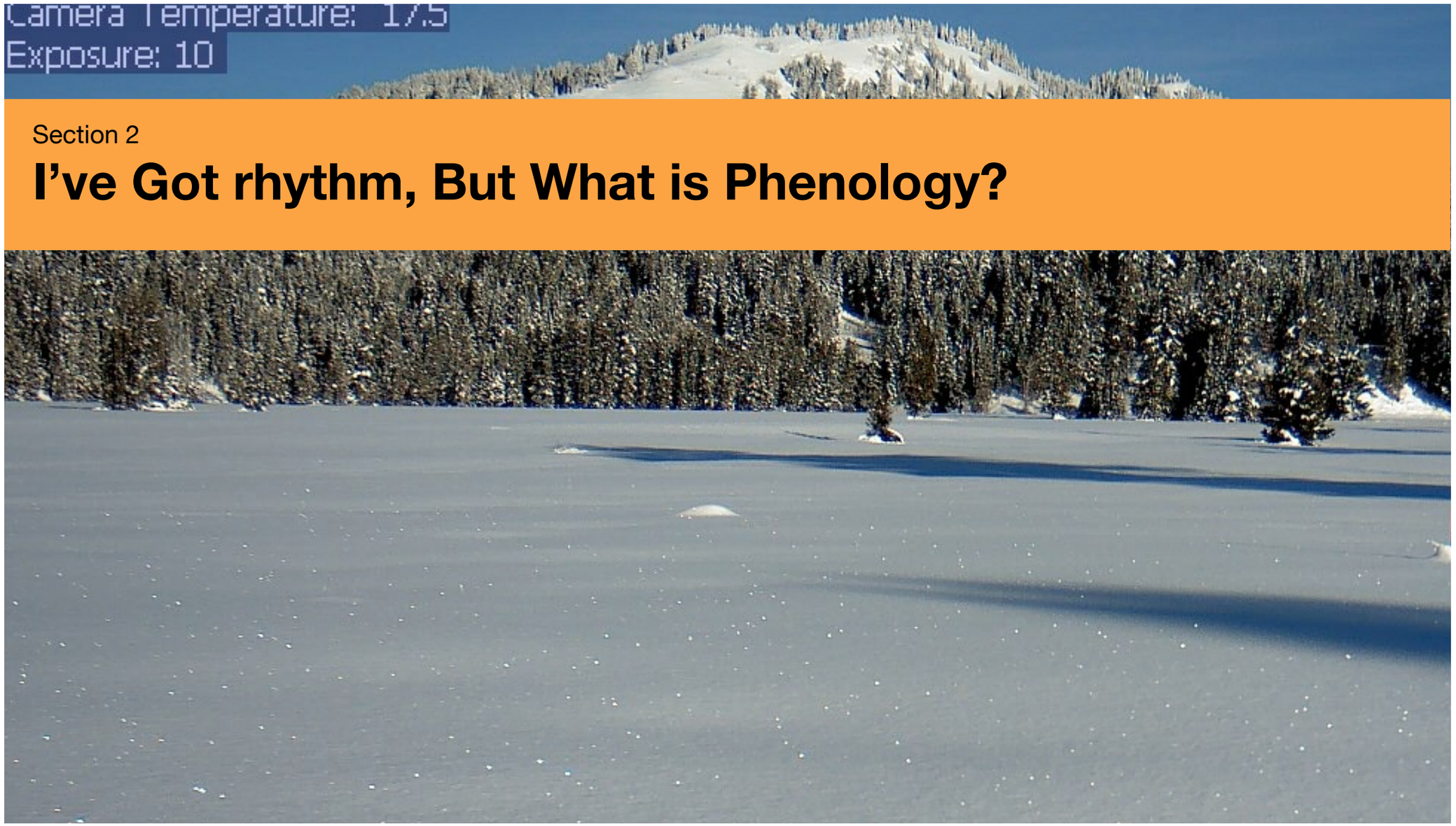
Parnassius taking cover until the sun comes out again.



Parnassius enjoying the sun, warming up and ready to take flight.

cloud cover changes the temperature and determines whether a butterfly is warm enough to fly or not. On rainy days, butterflies may not fly at all. Instead, they remain hidden in the vegetation waiting to fly until the sun comes out again. Sometimes they may have to wait more than one day until it warms up enough to fly.

Generally butterfly biologists say that if a person is comfortable in a short sleeved shirt, it is probably warm enough for *Parnassius* to fly, but if you have to keep your fleece on, the butterflies are probably staying cozy and hiding in a plant.



Section 2

I’ve Got rhythm, But What is Phenology?

Just as there can be differences in how hot or cold it is between two summer days, there are also differences in how snowy and cold it is between two years. This is environmental variation. The deep snow in the winter provides an insulating cover for the butterfly eggs, just like a blanket. When spring comes, and the snow melts,



Snowmelt on May 5, 2018.



Green up on May 23, 2018. A late spring freeze could damage Parnassius.

changing temperatures are important. If it gets too cold on a spring night, the eggs or caterpillars can freeze and die because there is no longer snow to protect them. So the seasonal variation, and rhythms, in weather patterns is described as **phenology** and is very important to *Parnassius.* Observe and compare the differences between the May 5th photo on the left to the May 23rd photo on the right, above. Can you list the differences and similarities you see?

Section 3

How Does Water Affect *Parnassius* habitat?



Parnassius live in dry meadows with gravelly soil. The water from rain or snowmelt drains quickly in this type of soil. Sagebrush *(Artemisia* species) are often present in meadows where we find *Parnassius.* But these meadows have to have enough moisture to support flowering plants too. *Parnassius* are more likely to be present if



Sagebrush among Balsmroot Arrowleaf.

there is a variety of flowering plants mixed in with the sagebrush (see food and foraging in Chapter 3, Section 2). If there are sagebrush, but no flowering plants, there will probably not be any *Parnassius* present.



Treeline along sagebrush meadow.

Parnassius can often be found in sagebrush meadows, but they also fly along the treeline. Treelines provide some protection from the wind, so this is an easier place to fly on a windy day.

Section 4

How Do You Track Phenology?



Butterfly Conservation Biologist, Dr. Diane Debinski next to a pheno-cam, which she uses to track seasonal changes in Parnassius habitat.

Phenology is the study of how seasonal patterns affect the timing of plant and animal emergence, blooming, egg laying, and other activities. A scientist might ask, when did the first lilac bloom in your town or when did the first robin appear? The timing of phenological events can vary across years. Scientists use data on

Dr. Debinski describes the value of the phenocam.



Dr. Debinski describes phenology.



phenological patterns across decades to track how climatic variation affects plants and animals.

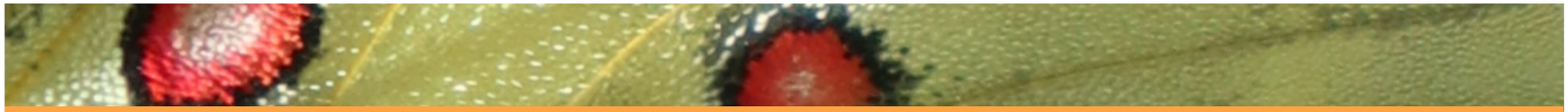
A phenocam is a camera that takes pictures of a landscape in the exact same place every day so that scientists can have images of the timing of the first green leaves, the first and last flowers, and the number of days when the meadow is covered by snow. This provides scientists with a way to see how many days in the summer flowers are available for *Parnassius* butterflies to nectar on. It also lets us know if there is a snow blanket to protect the *Parnassius* eggs in the winter and early spring.

When spring comes and the snow melts, the phenocam helps us predict when the butterflies will emerge. They usually come out when the Sulphur Flower Buckwheat begins to bloom.

Smile, you’re on Phenocam!



Phenocam Tracker. Tap the image above and then slide the dark gray rectangle on the yellow bar at the bottom of the screen. Track changes in the season that occur over time in the images captured by a phenocam (notice the dates at the top, on the left). Can you pinpoint the seasonal green up and when the first flowers bloom?



Section 5

Check Your Knowledge

Review 4.1

Why do we say that butterflies are “solar-powered”?

- **A.** Butterflies have small solar-powered cells on their wings that help them get warm.
- ✓ **B.** Butterflies need the sun to warm their flight muscles.
- **C.** Butterflies always fly upwards towards the sun.

Check Answer

Review 4.2

What is phenology?

- ✓ **A.** The study of how animals and plants respond to seasonal changes.
- **B.** The study of how plants produce pollen.
- **C.** The study of how butterflies fend off competitors.
- **D.** All of the above

Check Answer

Review 4.3

Why might a phenocam be useful to a scientist studying *Parnassius* butterflies?

- ✓ **A.** Phenocams can help determine if snowmelt conditions are appropriate for Parnassius eggs to have hatched.
- **B.** Phenocams can measure whether the wind speed is too high for butterflies to fly.
- **C.** Phenocams can help determine how many male and female butterflies are in a National Park.
- **D.** All of the above.

Check Answer

Review 4.4

What data could a scientist obtain from a phenocam that might help predict how weather could affect *Parnassius* population sizes?

- **A.** It could show the first day for the green-up of plants.
- **B.** It could help them track the first flowering of plants.
- **C.** It could help them compare the dates of snowmelt over a span of years.
- ✓ **D.** All of the above

Check Answer



CHAPTER
5

Wings That Make Waves

Making Waves, Taking Action



In this Chapter you will learn about the status of *Parnassius* in various locations and what scientists are doing to monitor and understand this species. Read on to consider how *Parnassius* is a *bioindicator* for climate change and pinpoint important ways you can participate in conservation.

Section 1

How Have Humans Impacted *Parnassius?*



Parnassius apollo in Pieniny National Park, Poland.

Between 1975 and 1999, *Parnassius apollo,* which occurs in Europe, went extinct in 10% of its range, and was in decline in 43% of its **range**. Populations were known to be stable in only 18% of its territory. Major factors causing the decline of the species noted by scientists studying *P. apollo* in Poland included extreme weather variability, broad impacts of industrialization, and butterfly over-



Dr. Mirosław Nakonieczny describes the recent history of *Parnassius apollo* in front of *Three Crowns Peak* in Pieniny National Park, in Poland.

Dr. Nakonieczny is the Head of the Department of Animal Physiology and Ecotoxicology at the University of Silesia in Poland. He works with Dr. Andrzej Kędziorski (Chapter 1, Section 2) to study the biology of *Parnassius apollo* in Poland.

collecting. These factors have reduced the population size, leading to reduced **genetic diversity**. The montane meadow habitat is also getting smaller due to forests growing in size.

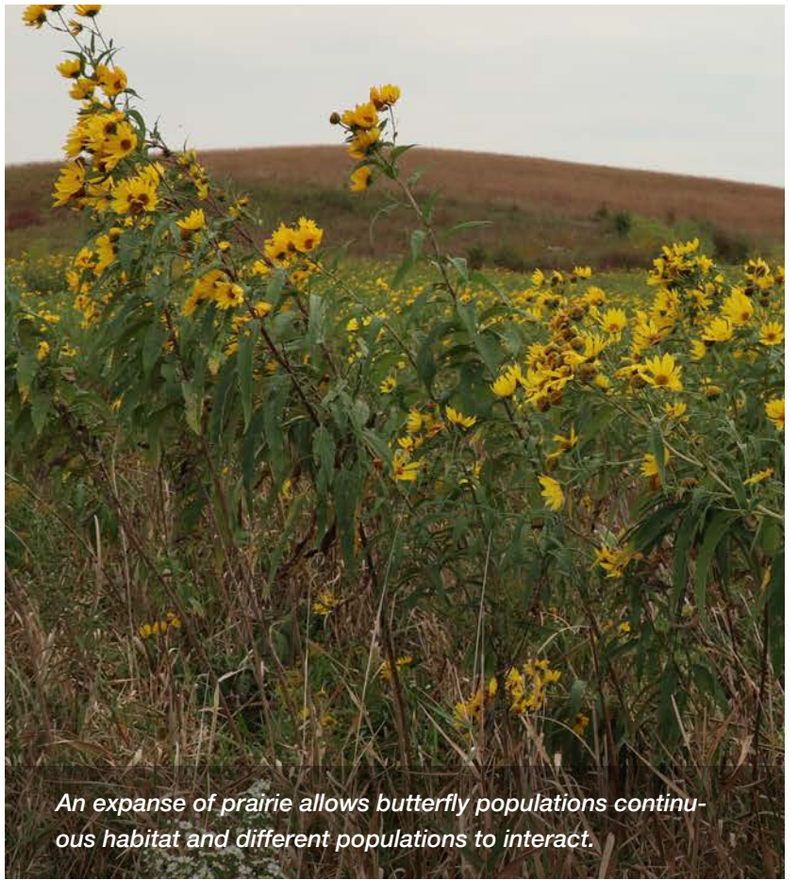
In Finland, intensification of agriculture may have reduced the suitability of habitat for the butterflies. In an area called the Sierra de Guadarrama in Spain, excess tourism and habitat destruction have created changes in the soil that have reduced the diversity of the plant community.

There have been several European studies examining the effects of heavy metal contamination of the soil and acid rain on host plants. Lead in the soil, which can come from burning coal for heating, could be taken up by the plant and when the caterpillars feed on the host plant they take in the metal contamination. These particular *P. apollo* butterflies depend on one species of host plant, *(Sedum tenuifolium),* and the adults use a very small number of nectar plant species, so they are particularly vulnerable to changes in the plants available.



Dr. Debinski describes the *Parnassius* butterflies in other parts of the world.

Montane meadow butterflies such as *Parnassius* that are constrained to isolated, shrinking meadow habitats are also highly vulnerable to **genetic isolation**, and for that reason could be under an increased extinction threat. Different populations of the same species usually exchange genes because of the dispersal and mating that occurs between these populations. If a population becomes isolated, there may be no dispersal to that population and thus no mating with other populations. This can affect the diversity of the genes in the population. If too much genetic diversity is lost, that can affect the ability for the population to reproduce and can put the population in danger of extinction.



An expanse of prairie allows butterfly populations continuous habitat and different populations to interact.



Connectivity in habitat can be disrupted by development.

So how do humans have a positive impact on *Parnassius* habitat? Some ways are protecting wild areas so that they are both large enough to support a population and so that there is connectivity between these areas. It is also important to minimize pollution and reduce emissions of greenhouse gases. Recycling, using fewer resources, helping to pro-

tect wild places, and getting involved in **habitat restoration** activities (such as Butterfly Waystations) are all things that you can do. You can also tell others about these delicate habitats and why they are important. Can you think of an insect habitat where you live that is important to protect? Maybe you've visited a pollinator garden, or even have grown one in your yard!

How Does Your Garden Grow?



Section 2

What Kinds of Conservation Efforts are in Action Now?



Across the globe scientists have become interested in *Parnassius* butterflies. *Parnassius* live in beautiful habitats and people who are interested in these habitats can't help but notice the butterflies. And as scientists, they are intrigued by the flurry of activity on a warm summer day.

But scientists have also noticed over the years that some of these populations, which were previously large in size, have become diminished. So scientists in Poland, Finland, Spain, Hungary, Japan, Mongolia, Canada, and the U.S. have studied *Parnassius* butterflies both from the perspective of conservation as well as

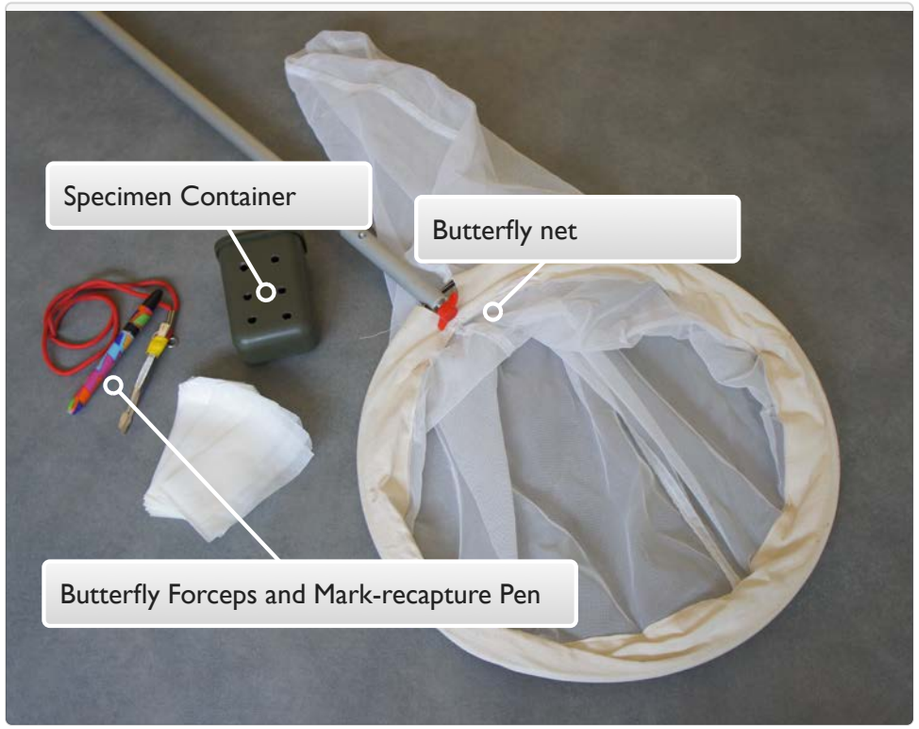
from the perspective of natural history, genetics, **systematics**, and **ecology**. Scientists across the globe are starting to compare notes and join together in their conservation efforts to try to understand what conditions are causing threats to *Parnassius* butterflies.

Dr. Diane Debinski, co-author of this iBook and a professor at Montana State University, has been studying *Parnassius clodius* in Grand Teton National Park for over 20 years. She has worked with several graduate students who have conducted research projects like those that you have learned about in this book as part of a Masters or Ph.D. degree.

Each year, Dr. Diane Debinski and her students, like Simone Durney (Ph.D. student) and Ben Sisson (undergraduate field assistant) featured in this book, collect a variety of data to help them study the effects of climate variation on the ecology of Grand Teton National Park, and specifically the *Parnassius* butterflies and their host and nectar plants. They measure items such as the height of all of the balsamroot

Butterfly scientists around the world are collaborating to understand the effects of climate change and how to protect Parnassius butterflies.

Explore the equipment used by butterfly researchers in the field. Click on each label below to learn more.



and Sulfur Flower Buckwheat nectar plants in the experimental plots every three days from the day that the plants emerge from the ground (late May) until they **senesce** (die) at the end of the growing season (mid-July). They can use these data to examine whether plots that had snow removed early or plots that have passive warming structures (photo, right) show different patterns of plant growth, emergence, or senesce than the control plots where no manipulation occurred. The team



Ben Sisson, undergraduate field assistant, is measuring the height of Arrowleaf Balsamroot. Ben is next to a ***passive warming structure****. This structure warms conditions underneath it without needing electricity.*

notes the first day that each of these species begins to bloom and when they senesce in the larger meadow using the phenocam images. They also count the number of days with snowcover in the meadow as a measure of the length of the winter season and conduct daily mark-recapture surveys of *Parnassius clodius* butterflies during the butterfly **flight season**.



Simone Durney is chasing Parnassius during her field study. She captures them temporarily to collect important data, and then releases the butterfly back in to the wild.



Dr. Debinski shows Ben the technique for mark-recapture, a procedure to only be performed by scientists so that the butterflies are not harmed.

Some of these data are called “experimental data” (data associated with an experiment) and some are called “observational data” (data associated with the natural world, but not collected in association with an experiment).



Dr. Debinski describes how her research team monitors *Parnassius* populations.

The experimental data are valuable in understanding how the nectar plants respond to less snowpack or warmer conditions in one particular year and in these particular experimental plots. The observational data are useful in understanding how long-term changes in butterfly and nectar plant abundance occur at a larger geographical scale and in relation to climatic variation.



Take a look at real phenocam data in Chapter 4, Section 4 of this book by using the “Phenocam Tracker” (p. 67). This will give you the opportunity to see how scientists use data to understand the natural world. In effect, you can be a public scientist.

Dr. Stephen Matter, a professor from the University of Cincinnati, has been studying *Parnassius smintheus* in the mountains of Alberta, Canada for over 25 years. He has been conducting mark-recapture studies similar to our research in Grand Teton National Park. Dr. Matter has found that *Parnassius* population sizes are affected by extreme weather events such as temperature and snowfall during the caterpillar’s overwintering period.



Parnassius is marked by scientists to be counted and tracked in their habitat. When recaptured, data on their range, flight season, and more can be recorded. The markings do not hurt the butterfly and only are done by trained scientists and field technicians.

For example, if there is a warm spell during early winter, the snow may melt, caterpillars may emerge too early, and would die from the cold temperatures after the warm spell. This would result in a lower number of butterflies observed during the following summer. Alternatively, if the insulating snow-



Ben records the number of plant species in measured plots. In other words, he is measuring species diversity.

pack is maintained and there are consistently cold temperatures when the caterpillars are overwintering, there may be less winter mortality and the number of butterflies observed in the summer may be larger.

The Debinski Team at Montana State University is now collaborating with Dr. Matter to compare whether the Grand Teton populations of *Parnassius clodius* show similar responses to weather patterns.

Because butterflies are relatively easy to study, identify, and monitor, they are some of the most well understood insects. So butterflies can help us understand what might be happening to other insects as well. They can be considered a “canary in a coal mine.” In other words, a decline in butterflies could make us aware that other less well known insects also may be in danger of extinction.



Section 3

How Do We Predict The Future of *Parnassius?*

The threat of climate change is a frightening topic. Many of us have seen movies or videos depicting how the globe has changed. It may be that summers are hotter, there is less snow at a ski area, or oceanfront properties may be experiencing higher water levels. It is difficult to imagine what one person can do about these large problems affecting the entire globe.

We also read about species going extinct in tropical rainforests and habitat loss. You may have even seen a new subdivision being built in your neighborhood where previ-

ously there was a forest or a meadow. As the human population increases, we encroach upon previously wild habitats, both directly and indirectly.

Direct impacts could include the building of a new subdivision or the replacement of the native plant community with a lawn. Both the soil compaction and the creation of a lawn remove the native host plant and nectar plants that butterflies need.

However, indirect impacts can occur as a result of the food we eat, the things that we buy, and the type of transportation we use. Much of the food that we buy in grocery stores comes from long distances. When we purchase food im-





ported from remote places, fossil fuel is used to transport that food hundreds or thousands of miles to the grocery stores. For that reason, there is quite a bit of interest in encouraging local food production and sales. How we live our lives affects the use of resources and the type of habitats that are available for species in the future.

Although these problems are large, and you may think of yourself as one small person, if lots of people make small positive changes, the results can be large.



We showed that the *Parnassius* butterfly is a small insect that can make big waves. Similarly, there are lots of things that you can do to have a positive impact. You and your family can plant a butterfly waystation in your yard or in a community garden. You could become a public scientist. You can also think about walking or biking sometimes rather than getting in a car if that is an option where you live. You can buy local food, recycle, and teach others about these ideas.

If you are intrigued by butterflies and want to become a public scientist, you can share your observations on websites such as iNaturalist and eButterfly.

When public scientists contribute their observations, the data available for research is increased. Sometimes public scientists

Many small positive changes can make a big difference

who have studied the same place for 10-15 years can contribute essential data that scientists can use to track population trends. So you as a public scientist can help contribute to the knowledge of *Parnassius* and other species where you live. Everyone can contribute to have a positive impact.

live nearby or visit a place where *Parnssius* butterflies live, we hope that you will keep your eyes out for them. But wherever you live, we hope that you notice the natural world and the plants and insects around you and think about conservation in your own neighborhood.

We hope that we have helped you learn about *Parnassius* butterflies, those flying polar bears, those small insects that make big waves. If you



Take your wings and Makes Waves

Section 4

Check Your Knowledge



Review 5.1

What threats to the survival of *Parnassius* occur in other countries?

- **A.** Climate change
- **B.** Habitat loss
- **C.** Chemical contamination
- **D.** Tree encroachment
- ✓ **E.** All of these

Check Answer

Review 5.2

What steps could you take to help protect *Parnassius* and other butterflies?

- **A.** Report sightings of the butterfly to eButterfly.
- **B.** Help protect large wild areas, such as montane meadows, to prevent genetic isolation.
- **C.** Plant a pollinator garden with plants that provide resources for the butterfly species of interest.
- ✓ **D.** All of these

Check Answer

Review 5.3

What is the value of doing science in the field?

- **A.** Scientists can't always simulate all of the conditions that are found in nature.
- **B.** Scientists could observe something unexpected that is a valuable clue in understanding natural history of a species.
- **C.** It's fun!
- **D.** It's a way to explore how the species of interest fits into the larger ecosystem.
- ✓ **E.** All of these

Check Answer



CHAPTER
6

Wings That Make Waves

B-Learning



Calling all Teachers!

Take what you've learned here further by implementing it in your classroom! Read on to see how you can connect this book to dynamic and fun educational activities that meet Next Generation Science and Common Core Standards. It's downloadable and free!

Now that you know a lot about our furry flying polar bear, *Parnaissius clodius,* you can take some steps to train as a public butterfly biologist! If you are a teacher, you can use this book and the lessons that follow in your classroom.

Download the PDFs linked herein to put together and customize your ***Parnassius* Teaching Kit!** Conduct surveys assessing student attitudes and ideas about *Parnassius* conservation and how local efforts connect to the global status of this butterfly, asking Qs like, "How does the rarity of *Parnassius clodius* in the Greater Yellowstone Ecosystem relate to butterfly populations in other countries?" Award your students with Scientists in-training Badges and take them into the field for hands-on activities that align with next generation science standards.

Our lessons include the following activities:

Lesson 1: Map a Classroom Exploration by Creating a Field Guide. This exercise helps students develop their skills as a keen observer of nature. This group or solo activity asks students to go outside, observe a particular aspect of the natural world (plants, fungi, insects, or birds, as well as clouds and weather), record observations, sketch, and compile their qualitative and quantitative observations into a classroom exploration field book. **Download here.**

Lesson 2: Build a Butterfly, Create Butterfly Character Cards. This exercise is intended to help students think about different evolutionary adaptations that help butterfly species survive. They will create a "Character Card" for a unique butterfly they create and choose its traits, such as wing color and size, habitat, and food preferences. Students will describe how the particular traits, such as large wings or nectar plant preference are advantageous to survival of that species. This lesson includes ***I Will Survive*,** a game which uses the Butterfly Character Cards created above to simulate how changes in habitat affect population growth and species survival. "Disturbances" occur as part of the game and students gain or lose "Survival Cards." At the end of the game, students evaluate which traits made their butterfly species more adaptable to changing environmental conditions. **Download here.**



Lesson 4: Phenology Tracker. This exercise allows students to use graphical and photographic **data from a real phenocam** in *Parnassius* habitat to evaluate seasonal patterns in the presence of snow and phenological patterns of flowering and green-up of the vegetation. The exercise asks students to think about how the timing of snowmelt affects the emergence date of the *Parnassius* butterfly and how changes across years could affect interactions between butterflies and their nectar plants. **Download here.**

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Credits

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Chapter 1, Section 1

pp. 3 and 4 Earth image: NASA

Chapter 1, Section 2

p. 8, Mountain image, p. 9, Dr. Andrzej Kędziorski image, and p. 10, Dr. Andrzej Kędziorski video: Dr. Mirosław Nakonieczny, Assoc. Prof. and Dr. Andrzej Kędziorski, Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, University of Silesia, Katowice, Poland.

Chapter 2, Section 1

p. 16, *P. apollo* image: Dr. Mirosław Nakonieczny, Assoc. Prof. and Dr. Andrzej Kędziorski

p. 17, *P. clodius* image: Simone Durney??

p. 20, *The Insect Body* app: Jordan Gongora

p. 21, Sexual dimorphism images: Simone Durney

Chapter 2, Section 4

p. 23, *P. apollo* image: Dr. Mirosław Nakonieczny, Assoc. Prof. and Dr. Andrzej Kędziorski

p. 29: Prudic, K. L., McFarland, K. P., Oliver, J. C., Hutchinson, R. A., Long, E. C., Kerr, J. T., & Larrivée, M. (2017). www.e-butterfly.org. eButterfly eButterfly: leveraging massive online citizen science for butterfly conservation. Insects, 8(2), 53. https://doi.org/10.3390/insects8020053

Chapter 2, Section 5

p. 32, *Sulfur flower buckwheat* image: Simone Durney

p. 33: images, Neal Smith Wildlife Refuge

Chapter 2, Section 6

p. 34, *Review 2.2* left images: Simone Durney

Chapter 3, Section 1

p. 43, *Dicentra uniflora* image: Simone Durney

Chapter 4, Section 1

p. 58, *Phenocam* image: B. Seyednasrollah, A. M. Young, K. Hufkens, T. Milliman, M. A. Friedl, S. Frolking, and A. D. Richard- son. Tracking vegetation phenology across diverse biomes using version 2.0 of the phenocam dataset. Scientific Data, 6(1):222, 2019. doi:10.1038/s41597-019-0229-9

Chapter 4, Section 2

p. 60, *Phenocam* image, as cited above.

p. 61, *Phenocam* images, as cited above.

Chapter 4, Section 3

p. 62, image: Simone Durney

Chapter 4, Section 4

p. 66, *Phenocam* images, as cited above.

p. 66, Phenocam app: Jordan Gongora

Chapter 5, Section 1

p. 69, image: Google Maps

p. 70, *P. apollo* in Pieniny National Park, Poland video: Dr. Mirosław Nakonieczny, Assoc. Prof. and Dr. Andrzej Kędziorski

p. 71, Dr. Mirosław Nakonieczny in Pieniny National Park video: Dr. Mirosław Nakonieczny, Assoc. Prof. and Dr. Andrzej Kędziorski

p. 73, lower left image: Simone Durney

Chapter 5, Section 2

p. 76, image: NASA Goddard

p. 77, butterfly equipment images: Simone Durney

p. 78, left image: Simone Durney and Ben Sisson

p. 81, image: Simone Durney

Chapter 5, Section 3

p. 82, image: NASA Goddard

p. 83, images: Diane Debinski

p. 84, image: Simone Durney

Chapter 6

p. 88, image: Andy Bartlette

CHAPTER SIX

CONCLUSION

Findings from this dissertation discovered that (1) extreme maximum temperatures during the spring led to decreased population growth rates of *Parnassius clodius* butterflies, (2) heating via passive warming and advanced spring snowmelt via snow removal led to earlier flower onset, extended flowering durations, and more flowers produced by arrowleaf balsamroot, and (3) there was no real effect of heating, but rather species-specific responses including extended flowering durations among yarrow and sticky geranium individuals, more flowers produced by sticky geranium individuals, and the greatest photosynthetic performance by yarrow followed by sticky geranium. Maximum soil moisture, soil temperature, air temperature, and surface temperature were the best predictors of photosynthetic performance. Specific findings for each research project are listed below. Additionally, the iBook science communication tool was presented at the 2022 GYE Biennial conference and the 2022 Entomological Society of America Conference as well as an art instillation featuring the iBook at the Museum of the Rockies in Bozeman, MT, USA since 2022.

Extreme maximum temperatures during the larval-pupal life stages were identified to have a significant effect on population growth rate, leading to a decline in the next generation of the population. We speculate that higher temperatures during the spring ephemeral host plant's flowering, and *P. clodius*'s larval stage, may lead to earlier plant senescence and lower *P. clodius* growth. Because *Parnassius* butterflies are well studied from a global perspective, results may aid in understanding the potential vulnerabilities of other insect species in montane environments to climatic changes. Findings from this study demonstrate the value in assessing a butterfly

species' response to short-term weather variation or long-term climatic changes *at each life stage* in order to protect and conserve insects and their interactions with other organisms.

The combination of heating with snow removal led to earlier flower onset, extended flowering duration, and increased maximum floral display. While there was year-to-year variation in floral phenology, the effect of heating with snow removal on earlier onset and maximum floral display strengthened over time. This suggests that short-term studies likely underestimate the potential for climate change to influence phenological plant traits. Overall, this research indicates that *Balsamorhiza sagittata*'s flowering onset responded more strongly to snow removal than to heating, but the combination of heating with snow removal allowed plants to bloom earlier, longer, and more profusely, providing more pollinator resources in spring. If warming and early snowmelt cause similar responses in other plant species, these patterns could mitigate phenological mismatches with pollinators by providing a wider window of time for interaction and resiliency in the face of change. However, it is important to remember that this is an early spring flowering plant species in a temperature-limited ecosystem. Later blooming plant species in this ecosystem or plants in other ecosystems may not be expected to respond similarly. This example demonstrates that a detailed understanding of how spring-flowering plants respond to specific aspects of predicted climatic scenarios will improve our understanding of the effects of climate change on native plant-pollinator interactions in montane ecosystems. Studies like this help elucidate long-term physiological effects of climate-induced stressors on plant phenology in long-lived forbs.

The effect of passive heating on the flower onset, flowering duration, maximum floral display, and photosynthetic performance (i.e., F_V/F_M) of yarrow (*Achillea millefolium*), sticky

geranium (*Geranium viscosissimum*), and littleflower penstemon (*Penstemon procerus*) individuals were monitored over the course of two consecutive growing seasons. Overall, the heating treatment had no effect, but species-specific responses were observed. Flowering durations of yarrow and sticky geranium were extended, and sticky geranium produced more flowers among the heating treatment. There was no effect of heating on photosynthetic performance, but species varied in photosynthetic performance with yarrow displaying the greatest photosynthetic performance followed by sticky geranium and littleflower penstemon. Decreasing mean photosynthetic performance was correlated with longer yarrow flowering durations and larger sticky geranium maximum floral displays. Maximum soil moisture, maximum soil temperature, maximum air temperature, and maximum surface temperature were the best local abiotic environmental condition predictors of photosynthetic performance. Yarrow and sticky geranium displayed tolerance to increasing drought conditions with longer flowering duration, more flowers produced and optimum photosynthetic performance. Therefore, we encourage investigation to include yarrow and sticky geranium in plant rehabilitation efforts, erosion control, and pollinator restoration strategies in the Rocky Mountain Region.

Ultimately, these findings contribute to existing knowledge concerning the responses of butterfly population dynamics, floral phenology, floral morphology, and flowering plant photosynthetic performance to changing and predicted environmental conditions in the Greater Yellowstone Ecosystem. This research highlights that consistent extreme warming events in the spring will likely contribute to decreasing population growth of environmentally dependent montane butterflies, specifically *Parnassiu clodius*. However, flowering plants in the Rocky Mountain Region seem to be responding positively to warming, and in some cases early spring

snowmelt, by flowering earlier, flowering longer, producing more flowers, and maintaining healthy photosynthetic performance. These findings indicate that floral communities are faring well under a changing climate, but butterfly counterparts may face declines. Overall, this could lead to cascading effects with declining butterfly populations eventually affecting floral populations due to reduced pollination services resulting in floral population declines.

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APPENDICES

APPENDIX A

EARLIER SPRING SNOWMELT DRIVES ARROWLEAF BALSAMROOT PHENOLOGY IN MONTANE MEADOWS

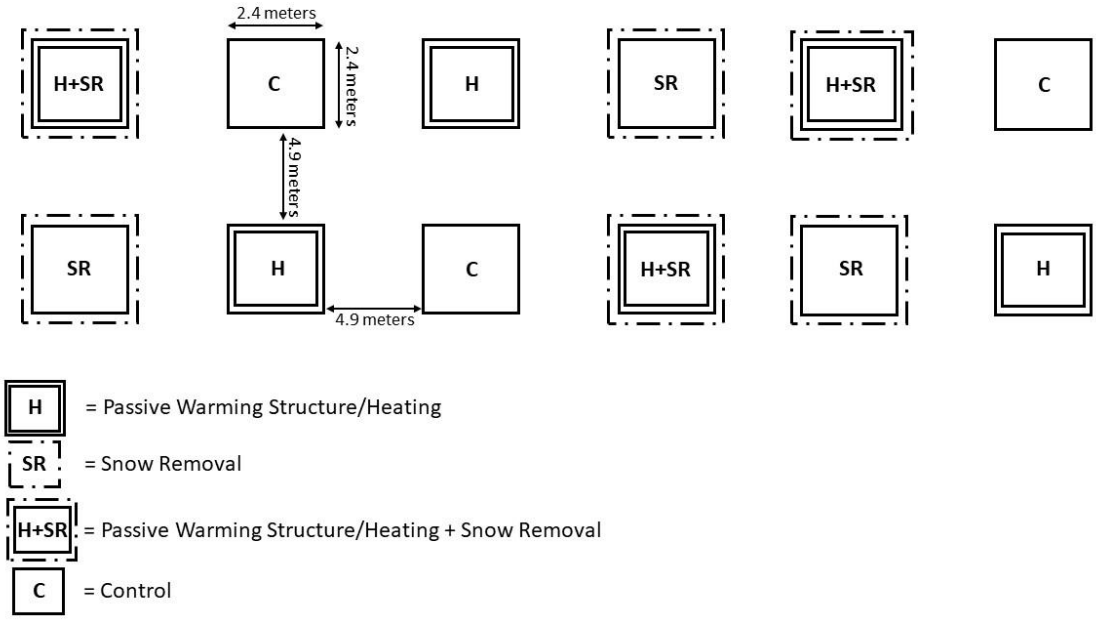


Figure A1: Schematic of the study area with plots of corresponding treatments of decreased spring snowpack via snow removal (SR: not heated, snow removal), warming via passive heating (H: heated, no removal), decreased spring snowpack and warming (H+SR: heated, snow removal), and control (C: not heated, no removal).



Figure A2: Individual *Balsamorhiza sagittata* with identification tag for continued monitoring.